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**PUSA**







# JOURNAL

OF THE

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VOLUME VIII  
1924-1925



1925  
ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS  
BOX 290, PENNSYLVANIA AVENUE STATION  
WASHINGTON, D. C.

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**THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS**

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## FIRST DAY.

### MONDAY—AFTERNOON SESSION—*Continued.*

#### REPORT ON INORGANIC PLANT CONSTITUENTS.

By A. J. PATTEN (Agricultural Experiment Station, E. Lansing, Mich.),  
*Referee.*

Because of the general revision of the *Book of Methods* that is now in progress, it has been deemed advisable to present at this time a brief review of the development of this chapter.

As many of the members know, it originated as a study of methods for analyzing "Ash" and was incorporated under "Soils". The associate referee or reporter on soils was usually delegated to carry on the work on ash. Very little was accomplished, however, until the two lines of work were divided, and at the 16th meeting a regular reporter was assigned to the subject of "Ash".

Following this action, excellent work was done for a number of years, but it was soon learned that, in burning plant materials in the usual manner, not all of the sulfur and chlorine and, in some cases, not all of the phosphorus or alkalis remained in the ash.

This resulted in the development of methods for the determination of these elements in the plant material itself rather than in the ash and it also necessitated a change in the title of the chapter. Therefore, at the meeting in 1901, by a vote of the association, the title was changed to "Inorganic Plant Constituents".

Owing to the manner in which this work has developed the entire chapter is now found to be in a rather unsatisfactory condition.

In the beginning of the chapter there is found a method for preparing the ash, which is used for the determinations of silica, iron and aluminium, calcium, magnesium, manganese, phosphoric acid, sulfuric acid, sodium and potassium, and chlorine. In addition, there are methods for determining the total amounts of potassium, sulfur, and chlorine in plants. Furthermore, the methods for the determination of iron and aluminium, calcium, magnesium, and manganese in the ash are not applicable for use on the ash of seeds. Also it is generally conceded that such determinations as carbon dioxide, phosphoric acid, sulfuric acid, sodium, potassium, and chlorine in the ash have very little significance since the results do not in all cases, if ever, represent the total amounts of these substances in the plant material itself.

The entire chapter, therefore, has been rewritten. Some of the methods have been deleted, and in cases where there are methods for

determining an element in the ash and in the plant material these have been combined so as to leave only one method suitable for determining the element in the plant.

The title of the chapter has also been given careful consideration. After conferring with a number of interested chemists the conclusion has been reached that the present title does not sufficiently define the present work included under this chapter and that which, in all probability, will be included under it in the future. The chief objection to the title at the present time is that the methods now included under this chapter call for the determination of the mineral elements in plants, which may exist either in inorganic or organic combination, while the title would indicate that they were intended only for determining those elements that were present in inorganic combination. However, there is a broader aspect to the study of plants than simply determining their mineral elements. For example, the Michigan Experiment Station is studying the effect of fertilizers on the storage of carbohydrates and upon the reaction of plant juices. The methods of analysis used in such studies, and any methods that may be developed in the future in connection with plant studies could be incorporated under this chapter if the title were changed. It has been suggested that "Plants" would be an appropriate title under which all methods used in the analysis of plants or plant substances could be included. The referee is heartily in favor of such a change.

The methods under this chapter as rewritten have been submitted to the Committee on Editing Methods of Analysis.

No report on sulfur and phosphorus in the seeds of plants was given by the associate referee.

## REPORT ON THE DETERMINATION OF IRON AND ALUMINIUM, CALCIUM AND MAGNESIUM IN THE ASH OF SEEDS.

By A. J. PATTEN (Michigan Experiment Station, East Lansing, Michigan.), *Associate Referee.*

During the past year little time was available for conducting further investigational work on this subject. Consequently, only the results obtained with the methods<sup>1</sup> as presented to the association last year can be reported.

The synthetic solution used for cooperative work was similar to the solutions used in former years and has the following composition:

---

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 421.

	Grams in 1000 cc.
CaO. ....	0.90
MgO. ....	1.80
FePO <sub>4</sub> .....	0.78
AlPO <sub>4</sub> .....	0.587
Mn <sub>2</sub> O <sub>4</sub> .....	0.06
K <sub>2</sub> O .....	5.34
Na <sub>2</sub> O .....	2.64
P <sub>2</sub> O <sub>5</sub> .....	8.60

Only three laboratories participated in the work this year. The results obtained are presented in the following table expressed as milligrams in 25 cc. of the synthetic solution:

Analyst	FePO <sub>4</sub> + AlPO <sub>4</sub>	CaO	MgO
1	33.6	22.1	41.1
2	33.8	23.2	42.9
3	36.0	23.1	42.1
4	38.5	20.5	40.8
5	37.8	19.4	..
6	36.9	..	..
Theory	34.2	22.5	45.0

These results do not speak well for the methods, which is hard to understand in view of the exceptionally good results received last year.

It is apparent from these figures that in all but two cases the high results for FePO<sub>4</sub> and AlPO<sub>4</sub> are due to contamination with calcium phosphate. This also causes low results for CaO.

In the investigational work on these methods, conducted in the referee's laboratory last year, it was found that calcium phosphate was not precipitated in the presence of ammonium acetate when the solution had a pH lower than about 7, but that the iron and aluminium phosphates were precipitated, under the same conditions, when the solution had a H-ion concentration corresponding to a pH of approximately 3.

In conducting this method the solution is first neutralized with dilute ammonium hydroxide until the indicator (thymol blue, acid range) just changes from red to yellow. This change occurs at pH 2.8. The ammonium acetate is then added, and the iron and aluminium phosphates should be precipitated free from calcium phosphate. The ammonium acetate solution used by the referee had a pH of a little over 5 but the amount added, 25 cc., did not in any case raise the pH of the solution above 3.

The referee has had some correspondence with W. H. Ross of the Bureau of Soils, U. S. Department of Agriculture, concerning this point, and experiences do not agree. In fact, his results are so different from anything obtained in the referee's laboratory that further time must be given to the investigation of the methods.

It is recommended that the methods for the determination of iron and aluminium, calcium and magnesium in plant materials be studied further.

## REPORT ON DAIRY PRODUCTS.

By JULIUS HORTVET (Minnesota State Dairy and Food Department, St. Paul, Minnesota), *Referee*.

No collaborative work has been carried on during the past year on the subject of moisture in cheese. The referee who had this subject in hand has not been able, owing to unavoidable circumstances, to continue the investigations that were reported two years ago. Attempts to secure the services of a referee to continue the work during the past year have not met with success.

The remainder of this report relates to the general subject of revising methods of analysis. It has always been considered by the present referee that one of his functions was to have in mind the subjects belonging under his group with a view to possible improvements in methods and the elimination of methods that are useless or that have gone out of practice. With this object in view, a letter incorporating an outline of contemplated changes in the status of some of the methods for the analysis of milk products was sent to a number of individuals. Some replies were received, and inquiries on this subject would have been continued had not another urgent matter demanded attention. This matter, relating to the importance of the Babcock test, was brought to the referee's attention in a communication from W. W. Skinner, enclosing a copy of a letter written by C. L. Alsberg, former chief of the Bureau of Chemistry, in which were related very fully the results of a conference held in California by a number of dairy science experts. It appeared to be the opinion in California that there was something wrong with the Babcock test as applied to milk, that the butter manufacturers were losing out, that they were not able to balance their factory output with the raw material purchased. A communication transmitted by C. F. Hoyt, in charge of the Dairy Laboratory of the California State Department of Agriculture, contains the following statement:

Results of considerable work done in the laboratories of the California Central Creameries and of the State Department of Agriculture \* \* \* show that percentages of fat in milk obtained by running the test according to directions in the standard method and reading the fat column in an 8% test bottle to the top of the meniscus are higher than those obtained by other methods, such as the Roese-Gottlieb, the Adams, and the asbestos. As the matter of accurate tests of milk by the Babcock method is one of very great importance to concerns engaged in the manufacture of dairy products, I would suggest that the matter of reading the fat column in the Babcock test of milk be brought before the appropriate referee of the Association of Official Agricultural Chemists.

The California investigators were inclined to advocate the use of glymol in testing milk in much the same manner as in testing cream.

The results of investigations reported by Hoyt<sup>1</sup> and Dahlberg<sup>2</sup> seemed to justify that practice. The referee, however, has been able, during the past year, to obtain from many sources valuable analytical data that warrant a contrary opinion, and therefore he is not able at the present time to recommend this modification in the Babcock test when applied to milk.

The Babcock method was devised by S. M. Babcock chiefly as a quick, practical method for factories, and after a period of careful investigations, sometime prior to 1908, was adopted by this association as official. For this reason it seemed urgent that an attempt be made to perfect the details to as high a degree as possible. Therefore, a statement, accompanied by a short questionnaire, was sent out under date of April 14, 1923, to about forty individuals. The statement reads in part as follows:

It is my aim to present at the next convention of the A. O. A. C. a description of the Babcock method for testing milk and cream to supplant the text that is now given in the Book of Methods<sup>3</sup>. There are several serious omissions in the description of the procedure and it seems to be a mistake to add at the close of Section 15—*Determination*, such a sentence as the following: "Details of the manipulation of the Babcock test and its application in the analysis of dairy products other than milk are described by Farrington and Woll, and Van Slyke". The authors to whom reference is made do not agree regarding the details of manipulation, and, as an illustration, attention may be directed to the disagreement regarding the measurement of the fat column. The entire procedure for carrying out the test, both for milk and cream, should be standardized and adopted by the A. O. A. C. as official, in order that we may have a uniform method which shall be binding in all parts of the country and which may be legalized in the various States. It is my hope that we shall be able to liberate ourselves from the laxities and disagreements existing among experts and others who are not only applying the Babcock test in connection with commercial establishments and laboratories but are also giving instructions to students. One important matter concerning which I desire your expression of opinion, backed up if possible by substantial analytical data, is the practice of using the so-called "red reader". I propose to obtain a full expression from as many experts as possible regarding this particular matter and hope to be able to make a definite recommendation in my forthcoming report. Another important point relates to the description of the 17.6 cc. pipet, 13 (C). There has been some discussion regarding this matter during the past year and there seems to be a difference of views on account of a conclusion reached some time ago by the U. S. Bureau of Standards. Just what is to be the standard pipet for use in the Babcock test ought to be finally decided. Also, it is important to decide the question relative to the 18 gram cream bottle *vs.* the 9 gram bottle for testing cream. Or shall both bottles be recognized? Attention should be given to the description of these test bottles with a view to correcting any defects that may now exist. There is also the question as to whether the standardization of Babcock glassware shall be referred to the U. S. Bureau of Standards or whether final action regarding those details shall rest with the Association of Official Agricultural Chemists.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 354.

<sup>2</sup> *Ibid.*, 7: 159.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 227.

The concluding sentence in the section relating to the present official Babcock method contains another objectionable feature. The reference to the application of the test in the analysis of dairy products other than milk and cream has for many years seemed objectionable. The inference that the test is applicable to such products as condensed milk, cheese, and ice cream is open to serious criticisms. A number of years ago, while the writer was serving as referee on this group of products, collaborative work covering three years resulted in the decision that no modification of the Babcock test is reliable when applied to cheese, condensed and evaporated milk, and ice cream. So far as the actual status of these modifications is concerned, in this association they are now in the refuse class. Therefore, the concluding sentence referred to should be omitted from the *Book of Methods*.

Not only have State experiment stations and other institutions issued bulletins describing the Babcock test, but State legislatures seem to have taken unwarranted liberties with important details of the method. For example, in Minnesota, there is a law the intent of which is that no other test bottle is legal except the 50 per cent, 18 gram, 6 inch bottle for testing cream. During the past year the U. S. Bureau of Standards issued revised specifications for Babcock glassware. The American Dairy Science Association has been actively at work on this subject for some time, with the result that there was issued on May 7, 1923, a document headed "Specifications for Babcock Glassware", published at the request of the Bureau of Standards. As a result of these investigations it seems important that this Bureau be recognized as the court of last resort on specifications for glassware. The American Dairy Science Association, in March, 1922, published a standard Babcock method that had been adopted by their committee headed by O. F. Hunziker.

Thirty or more replies to the circular letter were received from individuals in the United States and Canada. It was surprising as well as pleasing to discover the general interest regarding this subject. Most of the replies were of a high order and contained information of real assistance. As a contrast—a striking anti-climax, in fact, in comparison with the tenor of the correspondence in general—may be quoted the following, which constituted the chief item of information submitted by one of the State dairy commissioners:

The attitude of this department is that the law calls for accurate results and not merely the following of mere mechanical requirements.

The correspondence emphasized again the importance of a standard method, one that will be recognized by the leading associations. With their backing, those interested will be able to go forward safely. A number of controversial difficulties developed, relating, chiefly, to the following subjects:

- (a) The various types of cream test bottles to be adopted;
- (b) The construction of the 17.6 cc. pipet;
- (c) The accuracy of graduation to be required in the test bottles;
- (d) Methods of reading the tests;
- (e) The use of so-called red reader; and
- (f) Proper methods of sampling.

It will not be necessary to quote from the correspondence paragraphs relating to all these subjects, but the following will serve to illustrate the views regarding some of the most important questions under discussion. Two glassware manufacturers comment as follows on the subject of test bottles:

1.—In the copy of the A. O. A. C. specifications you enclose the 9 inch, 18 gram 50% bottle has been omitted, which I think is a mistake; it should be included since this bottle is an extremely popular one and the one I should use were I conducting a creamery. The 9 inch, 9 gram bottle is not a popular one judging from our sales, and this is due, I understand, to its being somewhat fragile because of the long neck of small diameter, giving a small sealing area where the neck is attached to the body and being harder to fill and empty, thus causing loss of time. The 6 inch, 9 gram 50% bottle is necessary because much of the cream tested runs over 50%, and about 90% of the testing machines in use today are made for the so-called 6 inch bottles. This is the mistake Wisconsin made—their largest 6 inch bottle is graduated to 40%. So what is the man going to do who gets 45 to 50% cream and his tester will not take a 9 inch bottle? For several years he was permitted to weigh out a 9 gram sample and double his reading, but because he did not have both the 6 inch, 18 gram 30% and 6 inch, 18 gram 40% bottle, he used his 40% bottles which, with the 9 gram sample, were equivalent to 80% bottles, and his results were far from being accurate; so this practice was barred by the State authorities. Now the operator must dilute his sample and, to my mind, this procedure in the hands of the average small creamery man is open to equally inaccurate results.

2.—You are perhaps familiar with the fact that at present there are over twenty different States in this country having different laws pertaining to the Babcock test. While these different laws do not vary to a marked degree, they require the manufacturer to break up his production and carry large stocks of these different bottles. The dealer in turn must do the same thing, and the result is that thousands of dollars worth of creamery glassware is tied up in stock; whereas if it were possible to standardize this glassware to four or five bottles that would absolutely take care of every need, there is no doubt that the cost of production of the milk and cream test glassware would be considerably reduced by this simplification. I do not know whether in a general standardization of bottles you can tie the users down to three types of bottles. This, of course, would be ideal. However, there is a big field to work on, as at present we manufacture at least one hundred different types and sizes of bottles to comply with the different State laws.

Much time was given to arguments, pro and con, relative to the milk pipet. As illustrative of the views presented by the correspondents the two following quotations are given:

1.—The facts in the case as I see them are as follows: In the 20th edition of Farington and Woll, page 45, it is made very clear why the 17.6 cc. pipet was adopted.



The prime object of the pipet is to deliver a charge of 18 grams of milk. The average specific gravity of milk is taken as 1.030, which means that 17.48 cc. (or as F. and W. say, very nearly 17.5 cc.) must be *delivered*. It is further clear that it was *found by trial* that about 0.1 gram of milk adhered to the walls of the pipet; therefore, the pipet was made to contain 17.6 cc. in order that it might deliver 17.5 cc. or 18 grams of milk. Now, a pipet with a *capacity* of 17.6 cc. and one *delivering* 17.5 cc. are one and the same thing, because they both deliver 18 grams of milk, which is all that we are interested in. If we wish to speak in terms of *delivery* instead of *capacity*, we can specify that the pipet should deliver 17.5 cc. and call it a 17.5 cc. pipet instead of a 17.6 cc. pipet; but I believe that the confusion that would result in the minds of thousands of testers, if the pipet were called a 17.5 cc., would more than offset any advantage of uniformity in the practice of calibration. I believe the pipet should continue to be called a 17.6 cc. pipet, that its capacity should be 17.6 cc. and its delivery 17.5 cc.

2.—To my mind, if we assume that the specific gravity of average milk at 20° C. is 1.0315 (in which case 17.45 cc. = 18 grams), what we want is a pipet graduated to deliver 17.45 cc. if filled and drained in the way universally approved for transfer pipets. To substitute, as is suggested, a pipet graduated to contain 17.6 cc., and to be blown out, involves the same assumption as regards specific gravity and, in addition, two other assumptions, neither of which can be relied upon. These are: (1) that any two pipets that contain the same volume will *deliver* the same volume, and (2) that any two pipets will be equally discharged when both are blown out. For we must keep in mind that when we calibrate a pipet to deliver, we adjust the mark until it does actually deliver the volume desired, when filled and discharged in the normal way for transfer pipets. Whereas, when we calibrate a pipet to contain, we adjust the mark until the instrument possesses a property of no direct value to us—its holding capacity; and then we make the unwarrantable assumptions that if it *holds* 17.6 cc., it will *deliver* 17.45 cc., and that, when blown out, it will always deliver the same volume. The fact that we do not know what the specific gravity of average milk is—assuming this to be a fact—militates in my opinion even more against the pipet to contain than against the pipet to deliver. In either case, if the specific gravity is not 1.0315, we make an error, and the error is the same in amount—provided the 17.6 cc. pipet actually delivers 17.45 cc. If it does not—and it is calibrated to contain, not to deliver, remember—then we have additional sources of error (as noted above) in the pipet to contain.

Relative to the revised specifications for the pipet, a statement from the Bureau of Standards, under date of February 24, 1922, reads as follows:

Under date of August 9, 1921, this Bureau sent out a letter to the various State Departments of Agriculture, Agricultural Colleges, and others interested in the use of the Babcock pipet for testing milk and cream, the purpose of which was to obtain an expression of opinion as to whether the pipet should be calibrated "to deliver" or "to contain" 17.6 ml. (cc.) of water at 20° C. A compilation of the replies received shows that the majority favor, and think it was the original intent of Dr. Babcock, that the pipet should contain 17.6 ml. (cc.) of water at 20° C. and that when so graduated it will deliver 18 grams of average milk. The charge of 18 grams is the basis for determining the fat content of milk and cream by means of the Babcock test. The Bureau made some determinations on the amount of milk delivered, and it was found that if the pipet contained 17.6 ml. of water at 20° C. the amount of milk (sp. gr. 1.032) delivered is much closer to 18 grams than if graduated to deliver 17.6 ml. of water. The milk remaining in the pipet tip after free outflow had ceased was in each case blown out. In consideration of the above, the Bureau would recommend that the

Babcock milk pipet be calibrated "to contain" 17.6 ml. of water at 20° C. and that the delivery time be from 5 to 8 seconds. At present some States require that the pipet "contain" and others that it "deliver" the 17.6 ml. of water at 20° C. We desire that you give the above your careful consideration in order that in the next revision of Circular No. 9 of this Bureau, a definite and uniform practice may be recommended which will be as satisfactory as possible to all concerned.

It developed in the course of the work during the past summer that it would be necessary to form a committee for the purpose of studying the material that had been gathered with reference to the Babcock method and agree upon a standard set of specifications for glassware and details in carrying out the test. After some correspondence a good committee was finally organized consisting of the following individuals: W. W. Randall and E. M. Bailey who, in cooperation with the referee, represented this association, and O. F. Hunziker, F. W. Bouska, and A. O. Dahlberg, representing the American Dairy Science Association. The committee met in Chicago on September 14th and 15th last, and spent a profitable time. The specifications on Babcock glassware adopted by the Bureau of Standards were reviewed, and the descriptive details of the method were discussed thoroughly. Randall served as secretary of the group representing this association and Bouska acted in the same capacity for the A. D. S. A. The committee adjourned during the afternoon of the 15th, and in due time thereafter the members received from Randall a very good report. This has been revised several times until now it represents the best efforts of the committee to formulate a standard Babcock method.

The amended form follows:

*Babcock method.*

I. APPARATUS.

(a) *Test bottles.*—The standard Babcock test bottles for milk and cream shall be as follows:

- (1) 8 per cent, 18 gram, 6 inch milk test bottle.
- (2) 50 per cent, 9 gram, short neck, 6 inch cream test bottle.
- (3) 50 per cent, 9 gram, long neck, 9 inch cream test bottle.
- (4) 50 per cent, 18 gram, long neck, 9 inch cream test bottle.

(1) *8 per cent, 18 gram, 6 inch milk test bottle.*—The total height of the bottle shall be 150-165 mm. (5.9-6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of milk for the bottle shall be 18 grams.

*Bulb.*—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm.; if conical, the outside diameter of the base shall be between 31 and 33 mm., and the maximum diameter between 35 and 37 mm.

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The contraction "cc.", throughout these specifications, refers to the true cubic centimeter—the volume occupied by 0.998877 gram of water at 4° C.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm. below the lowest graduation mark to at least 5 mm. above the highest. The top of the neck shall be flared to a diameter of not less than 10 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 8. The graduations shall represent whole per cent, five-tenths per cent, and one-tenth per cent, respectively, from 0.0 to 8.0 per cent. The tenths per cent graduations shall be not less than 3 mm. in length, and the five-tenths per cent graduations not less than 4 mm. in length and shall project 1 mm. to the left; the whole per cent graduations shall extend at least half-way around the neck to the right and shall project at least 2 mm. to the left of the tenths per cent graduations. Each whole per cent graduation shall be numbered, the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.20 cc. The maximum error of the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

(2) *50 per cent, 9 gram, short neck, 6 inch cream test bottle.*—The total height of the bottle shall be 150-165 mm. (5.9-6.5 inches). The bottom of the bottle shall be flat, and the axis of the neck shall be vertical when the bottle stands on a level surface. The charge of cream for the bottle shall be 9 grams.

*Bulb.*—The capacity of the bulb to the junction with the neck shall be not less than 45 cc. The shape of the bulb shall be either cylindrical or conical. If cylindrical, the outside diameter shall be between 34 and 36 mm; if conical, the outside diameter of the base shall be between 31 and 33 mm., and the maximum diameter between 35 and 37 mm.

*Neck.*—The neck shall be cylindrical and of uniform diameter from at least 5 mm. below the lowest graduation mark to at least 5 mm. above the highest. The top of the neck shall be flared to a diameter of not less than 15 mm. The graduated portion of the neck shall have a length of not less than 63.5 mm. The total per cent graduation shall be 50. The graduation shall represent five per cent, one per cent, and one-half per cent, respectively, from 0.0 to 50 per cent. The five per cent graduations shall extend at least half-way around the neck to the right; the one-half per cent graduations shall be not less than 3 mm. in length; and the one per cent graduations shall be intermediate in length between the five per cent and the one-half per cent graduations and shall project 2 mm. to the left of the one-half per cent graduations. Each five per cent graduation shall be numbered (thus: 0, 5, 10, — 45, 50), the number being placed to the left of the scale. The capacity of the neck for each whole per cent on the scale shall be 0.1 cc. The maximum error in the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

(3) *50 per cent, 9 gram, long neck, 9 inch cream test bottle.*—The same specifications shall apply to this bottle as to the 50 per cent, 9 gram, 6 inch cream test bottle, with the exceptions, however, that the total height of this bottle shall be 210-229 mm. (8.25-9.0 inches), that the graduated portion of the neck shall have a length of not less than 120 mm., and that the maximum error in the total graduation or any part thereof shall not exceed the volume of the smallest unit of the graduation.

(4) *50 per cent, 18 gram, long neck, 9 inch cream test bottle.*—The same specifications shall apply to this bottle as to the 50 per cent, 9 gram, 9 inch cream test bottle, with the exception, however, that the charge of cream for this bottle shall be 18 grams.

Each bottle shall bear on the top of the neck above the graduations, in plain legible characters, a mark denoting the weight of the charge to be used, *viz.*, "9 grams" or "18 grams", as the case may be.

Each bottle shall bear a permanent identification number, placed thereon either by the manufacturer or by the purchaser.

Each bottle shall be constructed of necessary strength to withstand the strain to which it will be subjected in the centrifuge.

The mercury and cork, alcohol and buret, and alcohol and brass plunger methods may be employed for the rapid testing of the bottles, but the accuracy of any questionable bottle shall be determined by calibration with mercury (13.5471 grams of clean dry mercury at 20° C. to be equal to 5 per cent on the scale of an 18 gram bottle and 10 per cent on the scale of a 9 gram bottle), the bottle being previously filled to zero with mercury.

(b) *Pipet*.—The standard milk pipet shall be as follows:

Total length: not more than 330 mm.

Outside diameter of suction tube: 6–8 mm.

Length of suction tube: 130 mm.

Outside diameter of delivery tube: 4.5–5.5 mm.

Length of delivery tube: 100–120 mm.

Distance of graduation mark above bulb: 15–45 mm.

Nozzle: straight.

Graduation: to contain 17.6 cc. of water at 20° C., when the bottom of the meniscus coincides with the mark on the suction tube.

Delivery: 5–8 seconds.

The maximum error in the graduation shall not exceed 0.05 cc.

The pipet is to be marked "Contains 17.6 cc.".

The pipet shall be tested by measuring from a buret the volume of water (at 20° C.) which it holds up to the graduation mark.

(c) *Acid measure*.—The device employed to measure sulfuric acid, whether a graduated cylinder or the pipet attached to a Swedish acid bottle, shall be graduated to deliver 17.5 cc.

(d) *Cream weighing scales*.—The standard cream scales shall have a sensibility reciprocal of 30 mg., *i. e.*, the addition of 30 mg. to either pan of the scales, when loaded to capacity, shall cause a deflection of the pointer of at least one division on the graduation. The scales shall be set level upon a stable support and be protected from drafts.

(e) *Weights*.—The standard cream test weights shall be 9 grams and 18 grams, respectively, and shall be plainly marked "9 grams" or "18 grams", as the case may be. They shall be made of material capable of resisting corrosion or other injury, shall preferably be of a low, squat shape, with rounded edges, and shall be verified at frequent intervals by comparison with standardized weights.

(f) *Centrifuge or "Tester"*.—The standard centrifuge, however driven, shall be constructed throughout and so mounted as to be capable, when filled to capacity, of rotating at the necessary speed with a minimum of vibration and without liability of causing injury or accident. It shall be heated, electrically or otherwise, to a temperature of at least 55° C. (130° F.) during the process of centrifugalizing. It shall be provided with a speed indicator—permanently attached if possible. The proper rate of rotation may be ascertained by reference to the table. By "diameter of wheel" is meant the distance between the inside bottoms of opposite cups measured through the center of rotation of the centrifuge wheel while the cups are horizontally extended.

Diameter of wheel, in inches . . . . .	10	12	14	16	18	20	22	24
Number of revolutions per minute . . .	1074	980	909	848	800	759	724	693

(g) *Dividers or calipers*, for measuring the fat column.

(h) *Water bath for cream samples*, provided with a thermometer and a device for maintaining a temperature of 38°–50° C. (100°–122° F.).

(i) *Water bath for test bottles*, provided with a thermometer and a device for maintaining a temperature of 55°–60° C. (130°–140° F.).

## II. CHEMICALS.

(a) *Commercial concentrated sulfuric acid*, of specific gravity 1.82–1.83 at 20° C. (68° F.).

(b) *Glymol*, or clear, white mineral oil, of specific gravity not to exceed 0.85 at 20° C. (68° F.). Oil-soluble artificial color may be added to the oil.

## III. COLLECTION AND PREPARATION OF SAMPLES.

(a) *Milk*.—The quantity of sample required will depend upon the number of determinations to be applied. For the usual analysis 250–500 cc. ( $\frac{1}{2}$ –1 pt.) of sample should be furnished; for the fat determination only, 50–60 cc. (approximately 2 fl. oz.) of sample will suffice. Single samples are to be preferred to composite samples. Bottled milk may be sampled by the collection of one or more bottles as prepared for sale. Composite samples shall be a product of not over one week and shall be tested as soon as possible.

Bulk milk shall be thoroughly mixed before the sample is withdrawn. This result is best accomplished by pouring the milk from one clean vessel into another three or four times. Where this is impossible, the milk shall be thoroughly and vigorously stirred for at least one-half minute with a suitable appliance long enough to reach to the bottom of the container. If cream has formed on the milk, the mixing shall be continued until all cream is detached from the sides of the vessel and evenly emulsified throughout the liquid.

Samples shall at all times be kept in non-absorbent containers, sealed air-tight, and held in the cold—but not allowed to freeze—until ready for examination. When transported by mail, express, or otherwise, the containers shall be filled, tightly stoppered, and marked for identification.

A necessary amount of preservative (corrosive sublimate, potassium bichromate, or formaldehyde) may be used, except in cases where the presence of preservative may be objectionable in connection with physical or chemical tests to be applied in addition to the determination of fat.

Before withdrawing a portion for test, the sample shall be poured into a clean empty receptacle and back until a homogeneous mixture is assured. Immediately before pipetting, the sample shall be brought to a temperature of 15°–20° C. (56°–68° F.). If lumps of cream or butter do not completely disappear, warm the sample to about 38° C. (100° F.) and mix thoroughly. Curdy and churned samples are not dependable.

(b) *Cream*.—The same instructions regarding collection of samples and use of preservative apply here as given under (a) *Milk*. Samples shall be tested as soon as practicable and not later than three days after they are taken. Composite samples representing portions of consecutive deliveries by the same patron are unreliable.

Immediately before testing, mix the sample by shaking, pouring, or stirring until it pours readily, and a uniform emulsion has been secured. If the sample is very thick, warm it to 30°–35° C. (approximately 85° F.) and then mix. In case lumps of butter have separated, heat the sample to 38° C. (100° F.) or, if necessary, to 50° C. (122° F.), by placing in a warm water bath. Mix thoroughly and weigh out at once. In commercial testing on a large scale, it may be advisable to warm all samples to 38°–50° C. (100°–122° F.) in a water bath previous to mixing. Great care should be exercised to avoid overheating the sample, thereby causing the cream to “oil off”. This precaution is especially necessary in the case of thin cream.

## IV. DETERMINATION OF FAT.

(a) *Milk*.—Transfer 18 grams of milk from the prepared sample to an appropriately marked milk test bottle by means of the pipet. The milk remaining in the pipet tip after free out-flow has ceased shall be blown out. Add 17.5 cc. of sulfuric acid,

preferably not all at one time, pouring it down the side of the neck of the bottle in such a way as to wash down any traces of milk into the bulb. The temperature of the acid shall be about 15°–20° C. (56°–68° F.). Shake until all traces of curd have disappeared; transfer the bottle to the centrifuge, counterbalance it, and, after the proper speed has been attained, whirl five minutes. Add soft water at 60° C. (140° F.), or above, until the bulb of the bottle is filled. Whirl two minutes. Add hot water until the liquid column approaches the top graduation of the scale and whirl one minute longer at a temperature of 55°–60° C. (130°–140° F.). Transfer the bottle to the warm water bath maintained at a temperature of 130°–140° F., immerse it to the level of the top of the fat column, and leave it there until the column is in equilibrium and the lower fat surface has assumed a final form. Remove the bottle from the bath, wipe, and with the aid of dividers or calipers measure the fat column, in terms of percentage by weight, from its lower surface to the highest point of the upper meniscus.

(b) *Cream*.—Weigh 9 grams or 18 grams, as the case may be, of the prepared sample, directly into an appropriately marked cream test bottle counterpoised on the weighing scales. Follow one of the following methods:

*Method 1*.—After the cream has been weighed into the test bottle, add 8–12 cc. of sulfuric acid, in the case of the 9 gram bottle, or 14–17 cc. of acid, in the case of the 18 gram bottle, or add acid until the mixture of cream and acid, after shaking, has assumed a chocolate-brown color. Shake until all lumps have completely disappeared and add 5–10 cc. of soft water at 60° C. (140° F.), or above. Transfer the bottle to the centrifuge, counterbalance it, and, after the proper speed has been attained, whirl five minutes. Add hot water until the liquid column approaches the top graduation of the scale and whirl one minute longer at a temperature of 55°–60° C. (130°–140° F.). Adjust the temperature, as directed under (a) *Milk*, and with the aid of dividers or calipers measure the fat column, in terms of percentage by weight, from its lower surface to the bottom of the upper meniscus.

*Method 2*.—(For a 9 gram bottle only.) After the cream has been weighed into the test bottle, add 9 cc. of soft water at 60° C. (130°–140° F.), or above, thoroughly mix, add 17.5 cc. of sulfuric acid, and shake until all lumps have completely disappeared. Transfer the bottle to the centrifuge, counterbalance it, and, after the proper speed has been attained, whirl five minutes. Fill the bottle to the neck with hot water and whirl two minutes. Add hot water until the liquid column approaches the top graduation of the scale and whirl one minute longer at a temperature of 55°–60° C. (130°–140° F.). Adjust the temperature and measure the fat column as directed in *Method 1*.

If *glymol* is used, introduce a few drops only into the bottle just before the reading is to be made; it must not be dropped in but must be allowed quietly to flow down the side of the neck. The surface separating the *glymol* and the fat is to be regarded as representing the upper limit of the column for purpose of measurement.

Whichever method is followed, the fat column for cream as well as for milk, at the time of reading, should be translucent, of a golden yellow to amber color, and free from visible suspended particles. All tests in which the fat column is milky or shows the presence of curd or of charred matter, or in which the reading is indistinct or uncertain, shall be rejected.

#### RECOMMENDATIONS.

It is recommended—

(1) That the standard Babcock method, agreed upon as a result of investigations carried on during the past year jointly with members of the American Dairy Science Association, including specifications for

Babcock glassware approved by the United States Bureau of Standards, be adopted as official to replace all methods and specifications heretofore adopted as official.

(2) That proposed changes and amendments to specifications for Babcock glassware be referred to the U. S. Bureau of Standards for investigation and approval.

(3) That proposed changes and amendments in the Babcock procedure for testing milk and cream be referred to the Referee on Dairy Products for collaborative study and investigation with instructions to report favorably or unfavorably, as the case may be, regarding such changes or amendments at the following meeting of this association.

(4) That this association cooperate in the study and perfection of methods for dairy products with the American Dairy Science Association, the American Public Health Association, and other organizations that may be interested in this subject.

(5) That further work be done on methods for dried milk and malted milk, including the use of the Jephcott modified Werner-Schmidt method.

No report on moisture in cheese was made by the associate referee.

## METHODS FOR FAT IN DRIED MILK.

By J. T. KEISTER (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The table accompanying this report includes results that have been obtained on comparative fat tests on dried milk by the regular Roesse-Gottlieb procedure with the use of ammonia, and the "neutral" procedure without the use of ammonia. It will be noted that in most cases a 10 percent solution of the sample was made up for these determinations.

The moisture was determined on the samples, and the fat was calculated on a water-free basis in each case. The results did not check in some instances as closely as desired, but most of the samples had been in storage a long time, and in some cases a satisfactory smooth fluid milk was not obtained; this no doubt accounts, in part, for the variations in the results.

It is noted by summing up the results on the five samples showing a higher percentage of fat by the neutral process that the average is 0.349, and that on the six samples with lower percentages by this process the average is 0.346. From these results it would be concluded that no advantage is gained in using the neutral method as far as the amount of fat extracted is concerned.

It would also be concluded that these results are not especially favorable to the use of a solution of the powder for weighing out portions for fat determination, although, as already stated, the age of the samples used may have accounted for the variable results-obtained.

Attention of the associate referee has been called recently to some work on fat determination in dried milk by H. Jephcott of England, in which he uses a mild hydrochloric acid treatment—modified Werner-Schmidt method—and claims to get good results. No opportunity has been afforded to try this out. It might be well to give it a trial. Jephcott had a copy of this paper with him on his recent visit to the World's Dairy Congress, from which other copies were made. This paper was published later<sup>1</sup>.

It is recommended that further study be made of methods for dried milk, including the Jephcott modified Werner-Schmidt method.

*Fat in dried milk—comparison of "neutral" method with the regular Roese-Gottlieb method, using a 10 or 15% solution by weight.*

SAMPLE NUMBER	WATER IN SAMPLE	FAT BY NEUTRAL METHOD*		FAT BY ROESE-GOTTLIEB METHOD*		DIFFERENCE†	REMARKS
		<i>per cent</i>	<i>Average</i>	<i>per cent</i>	<i>Average</i>		
14599	5.75	13.474 13.58	13.52	13.74 13.80	13.77	-0.25	15% solution used.
6478	2.55	0.987 0.887	0.937	0.975		-0.038	15% solution used.
19107	2.24	58.59 58.414	58.50	57.886 57.856	57.87	+0.63	10% solution used.
33602	3.64	25.539 25.358	25.448	24.954 24.92	24.937	+0.511	10% solution used.
9104	4.43	26.71 26.673	26.69	26.92 27.12	27.02	-0.33	10% solution used.
24901	5.22	1.20 1.276	1.238	1.118 1.156	1.137	+0.10	10% solution used.
26993	5.16	33.20 33.08	33.14	33.53 33.667	33.598	-0.458	10% solution used.
X	4.36	28.994 29.23	29.11	29.995	29.995	-0.88	10% solution used; sample "rough".
19110	3.68	12.32 12.42	12.37	12.40 12.52	12.46	-0.09	10% solution used.
14598	2.05	19.65 19.806	19.728	19.69 19.73	19.71	+0.018	10% solution used.
19109	3.91	27.911 27.945	27.928	27.37 27.51	27.44	+0.488	10% solution used.

\*Calculated to dry basis.

†Minus sign denotes lower results by neutral process and plus sign, higher.

<sup>1</sup> *Analyst*, 1923, 48: 529.



## DRUG SECTION.

### REPORT ON DRUGS.

By G. W. HOOVER (Bureau of Chemistry, Washington, D. C.),  
*General Referee.*

Though the referee has no comprehensive report to present, he should like to take this opportunity to state that in dealing with the subject of refining methods of analysis that are now available, by modification or otherwise, and that of the development of new methods for the analysis of drugs, the progress is necessarily slow; nevertheless, considerable work has been done. Full credit for such work as has been accomplished is due to the excellent assistance and support of the associate referees.

Owing to the nature of the work, an individual can give only a limited amount of time to the subject. It is obvious, therefore, that the more drug chemists that can be interested, the more rapid the progress will be. Practically the same associate referees have participated in this work for the past several years, but there is reason to believe that a number of pharmaceutical chemists who have not taken part would be willing to do so. It would seem desirable, therefore, for the future welfare of the work, to endeavor to secure the cooperation of additional associate referees to study specific subjects.

The referee wishes at this time to express his appreciation of the excellent work of the associate referees who have contributed so much during the past several years, and, finally, to emphasize what appears to be the most important point if the results that are so much needed are to be accomplished; that is, the expansion of this work by having more associate referees and covering a broader field.

### REPORT ON MERCURIAL TABLETS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

Most of the work done thus far has been devoted to the quantitative determination of mercuric chloride in antiseptic tablets. For the usual examinations it has been found that the iodometric method of E. Rupp<sup>1</sup> lends itself to the ready estimation of mercuric chloride, and this is the only volumetric method that has thus far been found useful.

The method is as follows:

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<sup>1</sup> *Chem. Zeit.*, 1908, 32: 1077.

Introduce 0.2 gram, accurately weighed, into a 250 cc. flask; dissolve in 25 cc. of water; add 2.5 grams of potassium iodide (dissolved in 5 cc. of water), 10 cc. of a 20 per cent solution of gum arabic, 30 cc. of normal caustic alkali or sufficient to make alkaline, and 3 cc. of a 37 per cent formaldehyde solution. Mix thoroughly and set aside for 10 minutes with occasional shaking. Add 10 cc., or sufficient to make acid, of 36 per cent acetic acid, diluted with an equal volume of water. Mix well, finally add 50 cc. of 0.1N iodine solution, stopper flask, and shake vigorously for 2 minutes, then occasionally until the mercury is dissolved.

Titrate the excess of iodine with 0.1N sodium thiosulfate solution, using starch as indicator. Deduct the excess of iodine from the total amount of iodine used, which gives the amount of iodine combined with the mercury. (1 cc. 0.1N iodine = 0.013575 gram Hg Cl<sub>2</sub>.)

The addition of the gum arabic solution is necessary to prevent the agglomeration of the metallic particles. When the operation was performed without the addition of the gum, the iodine attacked the mercury so slowly as to make the method impracticable.

Results by the Rupp method have been checked from time to time by the gravimetric method of weighing the mercuric sulfide after this had been dried and washed with carbon disulfide.

No trouble has been experienced in getting 100 per cent recovery from pure mercuric salts, but when these are incorporated in tablets the recovery is by no means so certain. It has been reported that tablets prepared with citric acid are not so easy to assay as other tablet preparations.

It is proposed during the coming year to study the accuracy of the Rupp method for mercuric chloride, both in the presence and absence of citric acid and other tablet constituents that might interfere with the determinations. Attention will also be directed to the determination of mercuric chloride in other preparations. Collaborative work will be solicited.

Acknowledgment is here made to J. F. Ellis, of the Bureau of Chemistry, for valuable assistance in making chemical analyses.

#### RECOMMENDATION.

It is recommended that a study be made of the influence of citric acid and other excipients on the Rupp method for mercuric chloride.

## REPORT ON METHODS FOR DETECTION AND DETERMINATION OF ADULTERANTS IN TURPENTINE.

By V. E. GROTLISCH (Leather and Paper Laboratory, Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The two methods for detecting and determining the extent of adulteration of turpentine with mineral oil (the usual form of adulteration), which were studied last year and adopted as tentative, were studied again this year. Samples were examined by four collaborators.

Both methods gave favorable and closely corresponding results. Neither gave strictly quantitative or theoretical results, owing to certain points of similarity in chemical properties between turpentine and the adulterants. Each set of samples consisted of a pure authentic gum turpentine and also the same adulterated to the extent of 3 per cent and 10 per cent, respectively, with a special mineral oil fraction used as a

### *Results of polymerization of turpentine containing known amounts of mineral oil.*

SAMPLES	ANALYST	FUMING SULFURIC ACID POLYMERIZATION RESIDUE			SULFURIC-FUMING NITRIC ACID POLYMERIZATION RESIDUE		
		Volume		Refractive Index at 20° C.	Volume		Refractive Index at 20° C.
A 3% Mineral spirits	1	cc.	per cent		cc.	per cent	
		0.12	2.4	1.4610	2.5	2.5	1.4388
	2	0.12		1.4501			
		0.16	3.2	1.4560	0.7*	0.7	1.4245
	3	0.14	2.8	1.4570			
		0.09	1.8	1.4690	2.6	2.6	1.4392
	4	0.09					
		0.08	1.6	1.4735	2.5	2.5	1.4310
		0.10	2.0				
B 10% Mineral spirits	1	0.36	7.2	1.4431	8.1	8.1	1.4380
		0.36		1.4401			
	2	0.34	6.8	1.4380	7.2	7.2	1.4290
		0.36	7.2	1.4385			
	3	0.30	6.0	1.4430	6.4	6.4	1.4290
		0.30					
	4	0.30	6.0	1.4450	6.0	6.0	1.4330
		0.30					
C Pure turpentine	1	0.08	1.6	.....	lost	.....	.....
		0.08					
	2	0.06	1.2	too little	none	0.0	.....
		0.06		to read			
	3	0.03	0.6	1.5130	none	0.0	.....
		0.03					
	4	0.04	0.8	1.5012	none	0.0	.....
		0.04					

\*Distillate too hot, due to inadequate condenser. Larger condenser used thereafter.

substitute for turpentine, known as mineral spirits or varnish makers' and painters' naphtha. Analysts are identified by number in the table as follows:

1. C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.
2. L. A. Salinger, U. S. Food and Drug Inspection Station, Savannah, Ga.
3. W. C. Smith, Leather and Paper Laboratory, Bureau of Chemistry, Washington, D. C.
4. V. E. Grotlisch.

The results of the determinations are shown in the table.

In addition to Samples A, B, and C, a series of samples containing as adulterant a coal tar product, solvent naphtha, was sent out for examination by the method published by Grotlisch and Smith<sup>1</sup>. No reports were made on these samples, and the referee is of the opinion that unless others than the authors of an analytical method put it to test no recommendations for adoption should be made. This determination is therefore held in abeyance for the present. It is hoped that this method can be studied next year.

#### RECOMMENDATIONS.

It is recommended—

- (1) That the tentative fuming sulfuric acid method for mineral oil in turpentine, described as Method I, be adopted as an official method.
- (2) That the tentative sulfuric-fuming nitric acid method for mineral oil in turpentine, described as Method II, be adopted as official.
- (3) That the Grotlisch-Smith method for coal tar oils in turpentine be further studied.

*Method I. Polymerization—Fuming sulfuric acid method.*

#### REAGENTS.

(a) *Fuming 38N sulfuric acid.*—Mix concentrated (95%) sulfuric acid with sufficient liquid fuming sulfuric acid to obtain a mixture containing slightly more than 82.38% total sulfur trioxide. If the fuming acid contains 50% excess sulfur trioxide, about 100 grams of fuming acid to 140 grams of concentrated acid will be sufficient. Determine the exact strength of the mixture and also of a reserve supply of concentrated acid as follows:

Weigh out a suitable amount of acid into a weighing bulb or pipet that has a capillary tube at the lower end and, at the upper end, a stopcock fitted with a platinum wire for suspending on the balance. Fill the bulb by slight suction, empty the lower end of the capillary by closing the stopcock simultaneously with the withdrawal of the capillary from the acid, and wipe off first with a moist and then with a dry cloth. Allow the acid to flow down the sides of the neck of a volumetric flask into a small quantity of cold water. If a flask having a volume of approximately 100 times the volume of the weighing pipet is used, the resultant solution will be near 0.5N. Wash

<sup>1</sup> *J. Ind. Eng. Chem.*, 1921, 13: 791.

all traces of acid into the flask, taking precaution to prevent loss of sulfur trioxide fumes. Make to volume and titrate from a buret against standard alkali. Calculate the sulfur trioxide content of both acids and add sufficient concentrated acid to the fuming mixture to bring it to 82.38% sulfur trioxide. After mixing, again determine the strength of the fuming mixture as before. The sulfur trioxide content of this acid must not vary more than +0.05% or -0.08% from the above value. The acid must be carefully protected against absorption of moisture from the air.

(b) *Concentrated sulfuric acid*.—95%, sp. gr. 1.84.

#### DETERMINATION.

Place 20 cc. of the 38N fuming sulfuric acid in a graduated narrow-necked Babcock flask, stopper, and place in ice water to cool. Add slowly, from a pipet, 5 cc. of turpentine. Mix the acid and turpentine, as added, by gentle shaking or rotation of the flask, keeping the temperature at about 60°–65° C. by continued immersion in the ice water. When the mixture no longer warms up on shaking, agitate thoroughly by shaking vigorously for about one-half minute. Place the flask in a water bath and heat at 60°–65° C. for 10 minutes, keeping the contents of the flask thoroughly mixed by shaking vigorously not less than six times during the heating period. CAUTION: If the shaking at first is too prolonged and vigorous, there is danger of the escaping sulfur dioxide forcing some of the mixture up over the mouth of the flask.

Cool to room temperature and fill the flask with concentrated sulfuric acid until the surface rises well up into the graduated neck. Centrifuge for 5 minutes at 1200 revolutions per minute, or for 15 minutes at 900 revolutions per minute, or allow to stand, lightly stoppered, for 12 hours. Read the unpolymerized residue (middle of meniscus), note the consistency and color, and determine the refractive index at 20° C.

Pure gum spirits of turpentine with this method gives a small residue, less than 2.0 per cent, which has a straw or darker color, viscous consistency, and a refractive index not less than 1.500. A limpid colorless residue with a refractive index less than 1.500 indicates adulteration with mineral oil. The unpolymerized residue from an adulterated oil represents from 60–80 per cent of the total quantity of adulterant present.

#### *Method II. Polymerization—Sulfuric-fuming nitric acid method.*

#### REAGENTS.

- (a) *Concentrated sulfuric acid*.—95%, sp. gr. 1.84.
- (b) *Fuming nitric acid*.—Sp. gr. 1.50.
- (c) *Concentrated nitric acid*.—Sp. gr. 1.42.

#### DETERMINATION.

Place 100 cc. of the sample in a 500 cc. Kjeldahl flask and distil in a current of live steam until 400 cc. of total distillate is collected. Transfer the distillate and the residue to separatory funnels and draw off the water. Return the oil separated from the distillate to the Kjeldahl flask, cool in ice water, and add slowly, with constant agitation, 50 cc. of concentrated sulfuric acid. Shake well to obtain complete reaction, keeping the flask cool. When the reaction is complete, again cool thoroughly and add 25 cc. of water. Distil the polymerized mixture in a current of live steam, collecting 900 cc. of total distillate. Separate the oily portion of this distillate and add to the oil sep-

arated from the first distillation residue. The condenser used in both distillations should be long and fitted with an adapter extending through a hole in a cover placed over the mouth of the graduate or other receiving vessel.

Place a volume of fuming nitric acid equal to three times the volume of the combined oils in a separatory funnel, and cool thoroughly in ice water. Add the combined oils, drop by drop, shaking carefully and keeping the mixture cool. After all the oil has been added, allow to stand quietly for a few minutes, until the reaction subsides. The mouth of the funnel should be fitted with a stopper carrying a long glass tube to act as an air condenser. Cool, draw off the acid, and wash the remaining oil once with a little fuming nitric acid, once with strong nitric acid, and finally several times with water. Measure the volume of the oil and determine the refractive index at 20° C. Pure gum spirits of turpentine gives little or no residue by this method.

## REPORT ON ARSPHENAMINE (SALVARSAN) AND NEO-ARSPHENAMINE (NEOSALVARSAN).

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee*.

In the report of 1922<sup>1</sup> it was recommended that the quantitative method for the determination of arspenamine and nearsphenamine, designated as Method No. 2, be adopted as tentative with the view to further study for final adoption.

The work this year has been a study of Method No. 2 in comparison with Method No. 1, which was adopted as official at the last meeting.

Samples consisting of ampules of neosalvarsan were sent to collaborators. The methods submitted were the same as those submitted last year<sup>1</sup>.

Results were reported by the following collaborators: H. Engelhardt and R. I. Grantham, Sharp and Dohme, Baltimore, Md.; G. W. Raiziss, University of Pennsylvania, Philadelphia, Pa.; C. G. Remsburg, U. S. Public Health Service, Hygienic Laboratory, Washington, D. C.; and C. K. Glycart.

*Results of determination of arsenic in nearsphenamine.*

COLLABORATORS	METHOD No. 1	METHOD No. 2
	<i>per cent</i>	<i>per cent</i>
Engelhardt . . . . .	18.95	19.04
	19.20	
Grantham . . . . .	18.70	18.48
Raiziss . . . . .	20.08	19.97
Rensburg . . . . .	19.96	19.69
	19.96	19.88
Glycart . . . . .	19.45	18.99
	19.45	18.75

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 461.

By comparison of the data it appears that the results of Method No. 2 are equally as good as obtained by Method No. 1.

#### COMMENTS BY COLLABORATORS.

*H. Engelhardt and R. I. Grantham.*—We wish to say again that we consider results obtained by Method No. 2 to be more reliable than those obtained by No. 1, in that Method No. 2 depends upon the direct titration of the arsenic present. The blank test in Method No. 1 is variable even when carried out under the same conditions, as the following examples show: 0.3, 0.8, 1.2, 0.7 and 0.9 cc. 01.N sodium thiosulfate required.

Because of the presence of a precipitate in the liquid in Method No. 2 we consider it advisable to use starch as indicator in the final titration.

*G. W. Raiziss.*—Method No. 2 seems to be more troublesome and I prefer the use of Method No. 1.

*Elias Elvove.*—Under the heading "Method No. 2"<sup>1</sup>, there is the statement "Applicable for assaying organically combined arsenic products". Now since the two methods are almost identical, with essentially the only difference between them being that whereas Method No. 1 ends with the titration of the iodine, in Method No. 2 the iodine is first titrated with sulfite and then the arsenious acid is titrated with iodine, it is difficult to see how this additional step would increase the applicability of the original method. Our own experience indicates that Method No. 1 is not applicable to all organically combined arsenic products. Thus, for example, a sample of sodium cacodylate which was known to contain about 33.5% of arsenic showed only about 4.3% arsenic by Method No. 1.

The comment by Elvove was referred to Grantham, who proposed Method No. 2, and the following reply was made:

I have not had occasion to determine arsenic in sodium cacodylate and would not say positively that this method would be applicable to this salt. We have not, up to this time, considered it necessary to include this test in our examination of this salt. Our standard is that it should conform to the U. S. P. requirements. I do know, however, that the method is applicable to atoxyl, because we have used it to a considerable extent and found it to be quite satisfactory (and I do not see any reason why it would not be applicable to sodium cacodylate as well). Since Dr. Elvove has raised this question, as a matter of interest I should like to make some experiments and will be able to forward my results within a few days.

#### RECOMMENDATIONS.

It is recommended—

(1) That Method No. 2 be adopted as official for the determination of arsenic in arsphenamine and neoarsphenamine.

(2) That a method be devised for the determination of arsenic in sodium cacodylate, since no assay is provided in the U. S. Pharmacopeia.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 463.

## REPORT ON LAXATIVE AND BITTER TONIC DRUGS.

By H. C. FULLER (Institute of Industrial Research, Washington, D. C.),  
*Associate Referee.*

In order to discuss the work and the results obtained during the past year, it will be necessary to refer to the report of the associate referee submitted at the 1922 meeting<sup>1</sup>.

## CASCARA SAGRADA ASSAY.

In 1922, when preparing the specimens to go to the collaborators for testing out the assay method, a good quality of ground drug was divided into two dozen parcels, each of which was placed in a screw-cap jar. About half of these specimens was submitted, and the others, unopened, were kept exposed to the light in a dry room.

The assays reported by the collaborators in 1922 were reasonably uniform, and the average per cent of total anthraquinones was 4.52.

The method submitted to the collaborators in 1923 differed but slightly from that previously sent out<sup>1</sup>. Some changes were made in the details as a result of suggestions by those who had worked with the method, and some obscure matters of phraseology were clarified.

The samples sent in 1923 were those that had been put up in 1922 and had not been used.

The results as reported up to the time of the meeting are shown in the table.

As will be noted, the results are reasonably uniform for any particular specimen that was tested by an individual collaborator, but there is a surprising lack of uniformity among the specimens themselves. The collaborators were chosen on account of their recognized familiarity with drug extraction and manipulation in general, and with this method of assay in particular.

It is not unlikely that a deterioration has taken place in the drug, and that in some packages the loss in anthraquinones has been greater than in others.

In order to verify this conclusion it is proposed to send out another set of specimens from a well-mixed batch of the same drug, of which there is a sufficient quantity in bulk still available.

Therefore, owing to the present anomalous situation, it is considered that another season may well be devoted to an intensive study of the method.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 7.



*Collaborative results on cascara sagrada assay.*

COLLABORATOR	TOTAL ANTHRAQUINONES			
	Gravimetric Method	Colorimetric Method		
		Direct	With Ammonia	
	<i>per cent</i>			
H. Engelhardt, Sharp and Dohme, Baltimore, Md.	1.86 1.86 1.82			
C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.	3.52  3.57	Yellow 3.50 Red 0.25 Yellow 3.80 Red 0.25	Red 2.20 Yellow 0.10 Red 2.10 Yellow 0.10	
H. C. Fuller	3.95  3.72  4.57	Yellow 9.4 ( $\frac{1}{8}$ -inch tube) Yellow 9.4 Red 0.54 Yellow 10.0 Red 0.68	Red 4.6 ( $\frac{1}{8}$ -inch tube) Red 4.6 Red 5.0 Yellow 0.6	
A. G. Murray, Bureau of Chemistry, Washington, D. C.	2.2			
P. J. Valear, Bureau of Internal Revenue, Washington, D. C.	2.37 2.37	Yellow 3.5 Yellow 6.4	Red 1.85 Red 3.4	
L. Burritt, Bureau of Internal Revenue, Washington, D. C.	1.78 2.71 2.92	Yellow 3.4 Yellow 6.8 Yellow 6.4	Red 1.85 Red 3.24 Red 3.0	
Average.....	2.80			

## ALLOIN ASSAY.

The results obtained by the method for determining aloin have been most gratifying when applied to the isolated substance. However, in attempting to apply it as an assay method for aloes the figures obtained have appeared to be too high and out of all proportion to the quantity of aloin that aloes are usually supposed to contain. This may be due to the fact that the drug contains a considerable quantity of a water-soluble constituent in addition to aloin that may be hydrolyzed to a substance that acts like the hydrolytic product of aloin. This point should be made the subject of further study. Whether this be true or not, it has been demonstrated by means of physiological tests, run in conjunction with the chemical assay, that aloes have a much greater laxative action than the pharmacopeial dosage would indicate. In other words, when compared with the specified dosage for pure aloin the actual dosage of aloes approaches more nearly the figure for the latter when considered in connection with its assay, even though the assay value may be greater than the published data on the subject would lead one to suppose had ever been found.

In conclusion, therefore, it is recommended—

(1) That further consideration be given to the method for determining anthraquinones in the laxative drugs of the cascara type.

(2) That further study be made of the applicability of the aloin method for the assay of aloes.

## REPORT ON ACETYLSALICYLIC ACID<sup>1</sup>.

By ARTHUR E. PAUL (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee*.

Last year's report on this topic included four recommendations, as follows:

(1) That last year's method for the determination of the melting point be adopted as a tentative method.

(2) That E. O. Eaton's idea of determining the melting point before and after crystallization from hot chloroform be investigated on pure and impure samples.

No work was done on these features during the present year. The referee understood that Eaton's suggestion was based essentially upon the belief that aspirin is not dispensed with other medicinal ingredients in tablet form. It was found, however, that tablets containing phenacetin, caffeine, and other substances, particularly laxatives, are being widely distributed.

(3) That last year's methods for combined and free acetic acid be further studied.

This work was not continued this year, for the reason that a careful review of the results previously submitted by collaborators did not indicate that the methods as submitted would ultimately prove satisfactory. Since the desirability of methods for these determinations is fully recognized, it is hoped that reliable procedures subsequently will be developed. For the present it seemed more desirable to devote some attention to the following recommendation:

(4) That the problem of determining aspirin in the presence of possible interfering substances be given consideration.

For this study a sample of "Aspirin Compound Tablets", containing acetylsalicylic acid— $3\frac{1}{2}$  grains, acetphenetidin— $2\frac{1}{2}$  grains, and caffeine— $\frac{1}{2}$  grain, was submitted to collaborators. This sample was designated as No. 2.

In addition to this work an article by William H. Gesell<sup>2</sup> was noted; it was stated that the volumetric modification suggested by the author

<sup>1</sup> Presented by W. O. Emery.

<sup>2</sup> *J. Am. Pharm. Assoc.*, 1923, 12: 228.

was found to be more satisfactory than the tentative gravimetric iodine method studied last year.

It was believed that Gesell's method should be given attention during this year, and accordingly Sample No. 1 was submitted to collaborators. This consisted of pure aspirin.

#### METHODS FOR SAMPLE No. 2.

##### *Separation and estimation of constituents.*

##### CAFFEINE.

Ascertain average weight of tablets. Dissolve 0.200 gram of the powdered tablets in a small beaker with 10 cc. of chloroform and filter into a 200 cc. Erlenmeyer flask. Wash the beaker with successive small portions of chloroform until the chloroform-soluble material is completely exhausted. Evaporate on a steam bath until the volume is reduced to about 10 cc. Add 10 cc. of sulfuric acid (1 : 10). Connect with a reflux condenser and digest for one-half hour with the flask partially immersed in a boiling water bath. Wash the reflux condenser with small amounts of chloroform and water. Cool, and transfer to a separatory funnel with a minimum amount of water so that the final volume does not greatly exceed 20 cc. Extract the caffeine and the salicylic acid with 5 portions of chloroform, using 30, 20, 10, 10, and 10 cc. Wash the combined chloroform extractions in a second separatory funnel with 5 cc. of water and add it to the aqueous acid solution, which is reserved for the determination of acetphenetidin. To the combined chloroform extractions add 20 cc. of water containing 1 gram of anhydrous sodium carbonate. Shake thoroughly. Transfer the chloroform layer to a second separatory funnel. Shake again with 20 and 10 cc. portions of chloroform. Wash the combined chloroform extractions with 10 cc. of water and add this wash water to the sodium carbonate solution. Filter the chloroform through a cotton pledget into a weighed beaker. Evaporate on the steam bath to a small volume and finish the evaporation without heat or with the aid of an air blast. Allow to stand in the air until the weight becomes constant. Calculate as Caffeine— $H_2O$  (U. S. P.).

##### ACETYSALICYLIC ACID.

Transfer the aqueous sodium carbonate solution to a glass-stoppered Erlenmeyer flask, using the minimum amount of water. Add 40 cc. of 0.1N iodine solution slowly from a buret. Heat gradually on a steam bath until the precipitate turns purple, loosening the stopper every few minutes to relieve the pressure. Let stand 15 minutes, cool, and acidify with dilute sulfuric acid. Shake to coagulate the precipitate. Filter quickly into an Erlenmeyer flask, washing the flask and filter paper with water until free from iodine. Titrate with 0.02N sodium thiosulfate. Each cc. of 0.1N iodine solution = 0.003001 gram of acetylsalicylic acid.

##### ACETPHENETIDIN.

Treat the phenetidin sulfate in the separatory funnel with small portions of solid anhydrous sodium carbonate until an excess remains at the bottom of the solution. Add 40 cc. of chloroform and 10 drops of acetic anhydride. Shake vigorously for 5 minutes. Continue the extraction, using 20, 10, 10, and 10 cc. portions of chloroform. Wash the combined chloroform extractions in a second separatory funnel with 5 cc. of water. Filter through a cotton pledget into a weighed beaker. Evaporate on a steam bath to apparent dryness, finally removing the excess of acetic anhydride by repeated additions of 1 cc. of chloroform and a drop of alcohol. Allow the reformed acetphenetidin to stand in the air until the acetic odor disappears. Desiccate over lime to constant weight.

## METHODS FOR SAMPLE No. 1.

*Comparison of last year's iodine method with modification suggested by Gesell.*

## LAST YEAR'S GRAVIMETRIC IODINE METHOD FOR TOTAL SALICYLATES.

Heat a 0.1 gram sample in a 200 cc. Erlenmeyer flask with 20 cc. of water and 1 gram of sodium carbonate on a steam bath for 15 minutes. Filter if necessary to remove talc. Dilute to 100 cc., heat nearly to boiling, add slowly 25–40 cc. of strong (about 0.2N) iodine solution—sufficient to insure an excess during digestion—and digest for an hour on a steam bath. Remove the free iodine with a few drops of sodium thiosulfate solution and decant the clear liquid through a tared Gooch, retaining most of the precipitate, tetraiodophenylquinon ( $C_6H_2I_4O_2$ ), in the flask. To the latter, add 50 cc. of boiling water, digest 10 minutes on the steam bath, filter, and wash gradually all the precipitate into a Gooch, using for this purpose and the final washing about 200 cc. of hot water. Dry to constant weight in an air bath at  $100^\circ C$ . Multiply the weight of the precipitate by 0.4016 to obtain the total salicylic acid and deduct the free salicylic acid previously determined. The difference represents the combined salicylic acid. Multiply by 1.304 to obtain the weight of aspirin.

## GESELL'S VOLUMETRIC MODIFICATION.

To 0.88 gram of acetylsalicylic acid, add 20.2 cc. of 0.5N potassium hydroxide, which is 0.2 cc. excess of amount required to convert to the potassium salts. Heat on a steam bath for half an hour. Transfer with water to a 250 cc. volumetric flask and make to volume. Pipet 25 cc. of the solution into a glass-stoppered iodine flask. Add 30 cc. of an approximately 0.1N sodium carbonate solution. Add slowly from a buret 33 cc. of 0.1N iodine solution. No precipitation should occur. Gradually heat on a steam bath until a precipitate forms and turns purple, loosening the stopper every few minutes to relieve the pressure. Let stand 15 minutes, cool, and acidify with dilute sulfuric acid. Shake to coagulate the precipitate. Filter quickly into a 500 cc. Erlenmeyer flask, washing the flask and filter paper with water. Titrate with 0.02N sodium thiosulfate. Each cc. of 0.1N iodine = 0.003001 gram of acetylsalicylic acid.

*Collaborative results on determination of acetylsalicylic acid.*

COLLABORATOR	SAMPLE No. 1. (pure aspirin)		SAMPLE No. 2. (aspirin compound tablets)
	Gravimetric Method	Volumetric Method	
	<i>per cent</i>	<i>per cent</i>	<i>grains per tablet</i>
H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.	99.0	99.5	3.460
	98.9	99.6	3.445
E. K. Nelson, Bureau of Chemistry, Washington, D. C.	99.44	99.44	3.343
	99.71	99.30	3.350
E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.			3.290
			3.720
C. W. Harrison, U. S. Food and Drug Inspection Station, Baltimore, Md.	98.4	98.53	
	98.97	98.4	
C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.	100.02	98.44	3.350
	100.28	100.26	3.362
Wm. Rabak, U. S. Food and Drug Inspection Station, Minneapolis, Minn.	99.50	99.42	3.351
	100.1	99.18	3.334
	98.84	97.73	3.238
	97.27		3.245

## COMMENTS BY COLLABORATORS.

*E. K. Nelson.*—In connection with the method submitted for the determination of aspirin in mixtures, the quantity of chloroform present during hydrolysis should be reduced to 2 cc. or less, whereby results approaching correctness are obtained.

In the volumetric method it should be stated at what temperature the assay should stand for 15 minutes. It was inferred that it should stand on the laboratory table, but better results are obtained by allowing to stand on the steam bath. Also, the use of 0.02N  $\text{Na}_2\text{S}_2\text{O}_3$  is not necessary—0.1N is accurate enough.

*C. W. Harrison.*—The methods of determining aspirin in mixtures are, as a whole, fairly satisfactory, except the separation of acetphenetidin and caffeine, since the method of hydrolysis is faulty, and also the caffeine is extracted from the solution very imperfectly. From my investigation it seems that the chloroform must have interfered in the hydrolysis.

*C. K. Glycart.*—In the analysis of the aspirin mixture, the acetphenetidin failed to hydrolyze under reflux. This must be due to the presence of chloroform.

The volumetric iodine method for salicylates appears to be no better than the gravimetric iodine method by comparison of my results—in fact, there is a tendency toward loss of iodine when the standard solution of iodine is manipulated according to the directions of the volumetric method.

## DISCUSSION OF RESULTS AND COLLABORATORS' COMMENTS.

In devising the methods for the separation of acetylsalicylic acid, phenacetin, and caffeine, the principal aim was to so arrange the details as to secure a satisfactory determination of the acetylsalicylic acid. Incidentally it was hoped that the same operation would also satisfactorily separate the caffeine from the phenacetin. While, however, the latter separation is incomplete, the determination of aspirin is considered fairly satisfactory.

Care must be taken in the hydrolysis of the original chloroform extract to guard against loss by volatilization. For this purpose the presence of a small amount of chloroform is desirable. On the other hand, acetphenetidin requires prolonged drastic hydrolysis, which is interfered with by the presence of chloroform. It does not seem desirable, therefore, to attempt to determine all three ingredients by a single hydrolysis. For this reason it would seem preferable to use one sample for the determination of aspirin and another for phenacetin and caffeine.

Regarding Gesell's criticism of the gravimetric iodine method and the comparative results given in the table, as well as the comments of the collaborators, it would seem that the volumetric method has no particular advantage. Furthermore, it involves filtration of an acidified iodine solution before titration, which is deemed to be objectionable. It is believed, therefore, that the original method should be retained.

## RECOMMENDATIONS.

It is recommended—

(1) That the following method for separating and determining acetylsalicylic acid in mixtures containing also caffeine and acetphenetidin be adopted as a tentative method:

Ascertain average weight of tablets. Dissolve 0.200 gram of the powdered tablets in a small beaker with 10 cc. of chloroform and filter into a 200 cc. Erlenmeyer flask. Wash the beaker with successive small portions of chloroform until the chloroform-soluble material is completely exhausted. Evaporate on a steam bath until the volume is reduced to about 2 cc. Add 10 cc. of sulfuric acid (1 : 10). Connect with a reflux condenser and digest for one-half hour with the flask partially immersed in a boiling water bath. Wash the reflux condenser with small amounts of chloroform and water. Cool, and transfer to a separatory funnel with a minimum amount of water so that the final volume does not greatly exceed 20 cc. Extract the caffeine and the salicylic acid with 5 portions of chloroform, using 30, 20, 10, 10, and 10 cc. To the combined chloroform extractions, add 20 cc. of water containing 1 gram of anhydrous sodium carbonate. Shake thoroughly. Transfer the chloroform to a second separatory funnel, wash with 10–15 cc. of water, reject the chloroform, and combine the sodium carbonate solution and wash water in a 200 cc. Erlenmeyer flask. Heat on the water bath to expel traces of chloroform, dilute to 100 cc., add slowly 25–40 cc. of strong (about 0.2N) iodine solution—sufficient to insure an excess during digestion—and digest for an hour on a steam bath. Remove the free iodine with a few drops of sodium thiosulfate solution and decant the clear liquid through a tared Gooch, retaining most of the precipitate, tetraiodophenylenequinon ( $C_6H_2I_2O$ )<sub>2</sub>, in the flask. To the latter, add 50 cc. of boiling water and digest 10 minutes on the steam bath; filter and wash gradually all the precipitate into a Gooch, using for this purpose and the final washing about 200 cc. of hot water. Dry to constant weight in an air bath at 100° C. Multiply the weight of the precipitate by 0.4016 to obtain the total salicylic acid and deduct the free salicylic acid previously determined. The difference represents the combined salicylic acid. Multiply by 1.304 to obtain the weight of aspirin.

(2) That the now tentative iodine method for total salicylates be adopted as official.

It is suggested that the determination of aspirin in the presence of other possible interfering substances, particularly laxatives, be given consideration, and it is hoped that next year's associate referee will succeed in devising satisfactory methods for the determination of free and combined acetic acid.

## REPORT ON METHODS FOR THE DETERMINATION OF PHENOLPHTHALEIN.

By SAMUEL PALKIN (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

In the associate referee's report of last year<sup>1</sup>, attention was called to certain inconsistencies in the results of two collaborators, G. C. Spencer on Method I, and O. L. Evenson on Method II. Although the other results were fairly concordant, it was deemed advisable to clear up certain points before recommendation for adoption was made. Accordingly, a further study of the steps involved in Method I (the iodination method) was undertaken to establish the following points: (1) whether or not the sulfite used for removing the excess iodine was causing hydrolysis or splitting off iodine from the tetra-iodo compound; (2) whether concentration of acid (HCl) was not a factor in the same direction; (3) to what extent dilution influenced the results; and (4) what advantages may be had by applying the iodination process to extracted residues of phenolphthalein rather than directly to the original sample. It was also considered essential to devise, if possible, some simpler means of estimating the reaction product, iodophenolphthalein, than by extraction.

As a result of the experimental work that was done in cooperation with A. G. Murray, Bureau of Chemistry, Washington, D. C., simplified directions were elaborated for the iodination method, in which the phenolphthalein is first removed from starch and other diluents by extraction with alcohol and the tetra-iodo compound is determined by filtration. Extraction of this compound by chloroform-acetone mixture is given as an optional procedure.

Method II—the ether-extraction method<sup>2</sup>—remains virtually the same as last year.

The sample submitted for cooperation was prepared by grinding and mixing about eleven different kinds of phenolphthalein tablets obtained in the open market from various manufacturers; they contained practically all of the flavoring agents and diluents commonly used.

Method I, as revised, and directions are as follows:

### PHENOLPHTHALEIN IN TABLETS.

#### *Iodination Method.*

When a solution containing phenolphthalein and excess iodine is made, alternately, alkaline to complete solution, and acid to complete precipitation, at low temperature, a quantitative yield of tetraiodophenolphthalein is obtained that may be filtered and weighed, or extracted by acetone-chloroform and weighed.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 14.

<sup>2</sup> *Ibid.*, 15.

## REAGENTS.

(a) *Potassium hydroxide solution*.—Dissolve about 100 grams of potassium hydroxide in an equal weight of water.

(b) *Hydrochloric acid*.—Concentrated.

(c) *Iodine reagent*.—Dissolve 20 grams of potassium iodide in a minimum amount of water. Add 14 grams of iodine and when dissolved dilute to 120 cc. Then add enough strong potassium hydroxide solution to discharge all the iodine.

(d) *Sodium sulfite solution*.—15%.

(e) *Solvent for optional determination*.—One volume of acetone to 3 volumes of chloroform (about 150 cc. for each determination). Both should be redistilled before mixing to insure freedom from residue.

## SAMPLE II.

(Sample I, sent previously, was of a simpler nature)

Sample consists of a mixture obtained from a number of various kinds of phenolphthalein tablets and contains sugar, starch, talc, calcium carbonate, flavoring agents, etc.

In the absence of other alcohol-soluble substances which are themselves, or which on treatment with iodine form compounds that are insoluble in aqueous acid medium or extractable by acetone-chloroform, including most plain tablets, as Sample II, count and weigh a suitable number of tablets to ascertain the average weight, grind to a *fine powder*, put through a No. 80 sieve, and mix thoroughly.

NOTE.—In the presence of fatty materials, etc., extractable by acetone-chloroform, particularly chocolate, subject the dry sample, finely ground, to a Soxhlet extraction with petroleum ether before applying the methods of determination.

## PREPARATION OF ALCOHOLIC EXTRACT.

Weigh out 1 gram of sample in a 100 cc. volumetric flask, add about 60 cc. of 95% alcohol, and boil gently for about 20 minutes, rotating the flask occasionally. Cool, make up to volume with alcohol, mix thoroughly, and filter through paper, covering the funnel with a watch glass to avoid evaporation. Pipet a number of aliquots (6 or more) of 10 cc. each into 100 cc. beakers, or preferably beaker flasks. Evaporate to dryness on the steam bath. If desired, one or more aliquots may be evaporated in tared beakers, the residues treated with a little ethyl ether, the ether evaporated, and the residues dried at 105° C. to constant weight. In the absence of other alcohol-soluble matter this gives the weight of phenolphthalein, and in any event gives a maximum value that is often desirable. The treatment with ether leaves the residues in a better physical condition for drying.

When ready for the determination, dissolve the dry residue obtained from the alcoholic extract in alkali, using about 5 cc. of water, to which has been added 3 or 4 drops of the potassium hydroxide reagent (a). Care must be taken to see that *all* of the phenolphthalein has gone into solution, as during evaporation a film of the residue is apt to creep up on the sides of the beaker. It is best to prepare the alcoholic residues separately, just before proceeding with the iodination.

## DETERMINATION.

*Conversion of phenolphthalein to iodophenolphthalein.*

To the alkaline solution of the phenolphthalein add a piece of ice (about 25 grams). Add sufficient iodine reagent (c) to insure an excess, the requirement for 0.1 gram of



phenolphthalein being about 3.5 cc. Add concentrated hydrochloric acid solution drop by drop from a buret to complete precipitation. If sufficient iodine has been added, the precipitate, as well as the supernatant liquid, will be brown; if it is not, add more iodine to insure an excess. Then add potassium hydroxide solution drop by drop from a buret, while stirring, to dissolve the precipitate completely and consume all the excess iodine. Repeat the process of precipitation with acid and re-solution with alkali 3 or 4 times with small amounts of the reagents, adding a small piece of ice, if necessary, to keep the solution cold. (In the acid condition there should be a brown precipitate resembling a periodide, and the supernatant liquid should be colored brown by the excess of iodine. The alkaline solution should be clear blue or purple-blue, and no precipitate should be present. In the presence of a large excess of alkali the solution may become decolorized or yellowish.) To the alkaline solution add enough sulfite solution (about 1 cc.) to insure consumption of excess iodine.

*Removal of iodophenolphthalein by filtration.*

Precipitate the iodophenolphthalein with hydrochloric acid, using about 1 cc. in excess. (This precipitate should be white. If it is brown, insufficient sulfite has been used. Redissolve with potassium hydroxide, add more sulfite, and reprecipitate with hydrochloric acid.) Allow to stand one-half hour, filter through a tared Gooch crucible, and dry in the oven at 100°–140° C. to constant weight. Multiply by the factor 0.3871 to obtain the amount of phenolphthalein.

*Removal of iodophenolphthalein by extraction—(Optional).*

Transfer the alkaline solution containing the iodophenolphthalein, with washings, to a 200 cc. separatory funnel. Add an equal volume of solvent; acidify with hydrochloric acid, using about 1 cc. in excess; and extract. Wash the solvent layer successively through two separatory funnels, each containing 20 cc. of water, finally drawing it into a tared Erlenmeyer flask. Follow this by three additional extractions, using 25 cc. of solvent for each. Wash each of these solvent layers successively through the two separatory funnels containing the 20 cc. of water, finally drawing them into the tared Erlenmeyer flask.

In transferring the *first extract*, which is the most concentrated, through the successive funnels and ultimately to the flask, use a few cc. of fresh solvent to wash the funnel stem. Do not filter the extracts as the iodophenolphthalein will crystallize out on the filter, in which form it is very difficultly soluble in chloroform-acetone. The two successive washings with water can be depended upon to remove salts, etc. Evaporate the combined solvent extracts on the steam bath, using an air blast if available. Evaporate the last 20 cc. below the boiling point to avoid loss by decrepitation. Dry the residue in an oven at 105° C. for 20–30 minutes to constant weight. Multiply the weight of iodophenolphthalein by the factor 0.3871 to obtain the phenolphthalein content.

## EXPERIMENTAL WORK.

The data recorded in Tables 1, 2, and 3 are given to show the effect of varying aqueous dilutions (with respect to concentration of reaction product) and concentration of hydrochloric acid and sodium sulfite, respectively, on the quantitative return of iodophenolphthalein.

In all experiments of a given series, care was taken to maintain all other conditions constant while tracing the effect of the variable, and more drastic variations were introduced for each variable than would

ordinarily be encountered in practical application of the method. As shown in the tables, reasonable variation in the concentrations of acid and sulfite, as well as in the concentration of iodophenolphthalein with respect to all other conditions combined, is practically without effect on the accuracy of the method.

The designations A, B, and C refer to the respective solutions of which aliquots were employed in the experiments. These, in all cases, are solutions of iodophenolphthalein that have been brought, as a whole, through the various steps in the iodination process, up to the step to be tested and diluted to a given volume. In this way conditions for the nonvariants could be maintained absolutely constant.

TABLE 1.  
*Variation in aqueous solution.*

SOLUTION A*	H <sub>2</sub> O ADDED	CONCEN- TRATED HCl USED	TOTAL VOLUME	WEIGHT OF IODOPHEN- OLPHTHALEIN	PHENOLPHTHAL- EIN CALCULATED	RECOVERY
cc	cc.	cc.	cc	gram	gram	per cent
25	25	1	51	0.1277	0.04943	98.86
50	25	1.5	76.5	0.2562	0.0992	99.2
50	50	2	102	0.2571	0.0995	99.5
50	.....	1	51	0.2573	0.0996	99.6

\*0.5 gram of phenolphthalein converted to tetraiodophenolphthalein by Method I and diluted to 250 cc.

TABLE 2.  
*Variation in hydrochloric acid.*

SOLUTION B*	CONCENTRATED HCl	WEIGHT OF IODOPHENOLPHTHALEIN	WEIGHT OF PHENOLPHTHALEIN CALCULATED	RECOVERY
cc.	cc.	gram	gram	per cent
25	1	0.1130	0.0515	99.5
50	1	0.2677	0.1033	99.7
50	2	0.2669	0.1033	99.7
50	4	0.2668	0.1032	99.7
50	8	0.2675	0.1035	99.8

\*0.5176 gram of phenolphthalein converted to tetraiodophenolphthalein by Method I and diluted to 250 cc.

TABLE 3.  
*Variation in concentration of sulfite.*

SOLUTION C*	SODIUM SULFITE SOLUTION	CONCENTRATED HCl	WEIGHT OF IODOPHENOL- PHTHALEIN	WEIGHT OF PHENOLPHTHALEIN CALCULATED	RECOVERY
cc.	cc.	cc.	gram	gram	per cent
25	1	1	0.1263	0.04889	87.78
50	1	1	0.2555	0.0989	99.78
50	2	1	0.2555	0.0989	99.78
50	4	1	0.2580	0.09987	99.74
50	8	1	0.2563	0.09921	98.42

\*0.5 gram of phenolphthalein converted to tetraiodophenolphthalein by Method I and diluted to 250 cc

TABLE 4—Results obtained by collaborators.

COLLABORATOR	SAMPLE II.				SAMPLE I.				METHOD II.	
	METHOD I—Iodination Method.		METHOD II.		METHOD I.		METHOD II.		By extraction with ether	
	By Gooch filtration of iodophenolphthalein	per cent	By extraction of iodophenolphthalein with chloroform-acetone	per cent	By Gooch filtration	per cent	By extraction with chloroform-acetone	per cent	By extraction with ether	per cent
W. F. Kunke, Bureau of Chemistry, Washington, D. C.	33.6					69.68				
	33.6					70.18				
	33.48					69.52				
	33.45					69.64				
	33.21					70.18				
G. C. Spencer, Bureau of Chemistry, Washington, D. C.	33.78									
	33.44					72.32		70.29		71.0
	33.00					69.68		69.4		73.5
	33.55					70.77		69.08		73.2
						70.13		68.50		
C. D. Wright, Bureau of Chemistry, Washington, D. C.						69.57		69.8		
						69.01		68.50		
	33.0 } 10 cc. aliquot									
	33.1 } 10 cc. aliquot									
	33.7 } 20 cc. aliquot									
E. K. Nelson, Bureau of Chemistry, Washington, D. C.	33.3 } 10 cc. aliquot									
	33.7 } 10 cc. aliquot									
	(1.5 gm. sample 100 cc.)									
	34.02									
	33.99									
S. Palkin.										
	33.79 (0.1 gram)					69.7		69.7		
	33.79 (0.1 gram)					70.0		70.06		
	33.56 (0.1 gram)					69.7		69.7		
A. G. Murray.	34.0 (0.05 gram)					70.06 (0.1 gm.)		70.5		
	33.72 (0.250 gram)					69.68 (0.2 gm.)		70.6		
	34.06 (0.1 gram)									
	34.1 (0.2 gram)									
	33.86									
	33.64									
	34.26									
	34.34									
	33.37									
	33.18									
	34.03									
	34.25									
	30.24									

Table 4 shows the results obtained by the collaborators. Sample I is a part of the material used in last year's cooperative study. Reports on this sample were made by several of the collaborators who wished to test out the revised procedure (Method I) on that sample.

It will be seen that, in the main, the results of the different collaborators are fairly concordant. The results of each of the *individual* collaborators do not check quite so well in some cases.

The few results reported on Sample II by the optional procedure (extraction with chloroform-acetone) show consistently higher values by about 1 per cent over those obtained by filtration. A petroleum-ether extraction of Sample II showed that it contained about 5.5 milligrams of non-volatile petroleum-ether-soluble matter per 5 gram sample. This residue was further augmented, no doubt, by non-volatile matter in the chloroform-acetone solvent, if these reagents were not previously redistilled. Some of the non-volatile petroleum-ether-soluble matter from excipients and flavoring matter, thought to be negligible in this sample, is evidently sufficient to affect appreciably the results obtained by the extraction procedure.

The collaborators, with the exception of Murray, reported on Method II; not so many determinations were made, however, as by Method I. In practically all cases the ether extraction method yields higher results (approximately 2 per cent) than the iodination method. This method would still be valuable, however, as a check in conjunction with other methods, particularly where interfering substances are known to be absent.

#### RECOMMENDATION.

It is recommended that Methods I and II be tentatively adopted, and that they be further studied with regard to their application to a larger variety of drug products.

*G. W. Hoover:* We have probably all observed that within recent years a number of preparations claiming to possess radioactivity has been introduced upon the market. Reference is made particularly to preparations intended for oral administration. There are also upon the market certain waters represented to possess radioactivity. J. W. Sale of the Water and Beverage Laboratory, Bureau of Chemistry, has done considerable work upon the subject of methods for the examination of waters represented to possess radioactivity, and I am going to ask Mr. Sale if he will be good enough to discuss the subject informally at this time, particularly regarding the methods developed or employed by him in the examination of these products.

*J. W. Sale:* In 1916 there was presented to the association a report on the determination of radioactivity<sup>1</sup> "with the hope that eventually uniform procedures for the measurement of radioactivity may be adopted by the association". Since that time, a number of drug preparations has appeared on the market. These include pills, bath compounds, hair tonics, ointments, cosmetics, suppositories, and various solutions alleged to contain radium or radium emanation, and therefore it is now even more desirable than it was in 1916 for the association to adopt a standard procedure for the determination of radioactivity. Briefly, the determination of radium or radium emanation that is present in a clear solution consists in boiling out the emanation, which is a gas, and determining its effect upon the rate of fall upon a gold leaf in a charged electroscope. There are, of course, a number of factors to be considered if accurate results are to be obtained. When the sample consists of a solid, semi-solid, or liquid containing suspended matter, a clear solution must be obtained by some means before proceeding with the analysis, and different kinds of samples require different treatments. It is our custom to report content of radium in terms of grams radium per gram of sample, or if the sample is a liquid, such as a mineral water, as grams radium per 1 cc., 100 cc., or 1 liter of sample. If the quantity of radium is very small, as it usually is, the unit used is the millimicrogram, which is one-billionth of a gram. Radium emanation is reported in terms of curies per 1 cc., 100 cc., or 1 liter of liquid, or if the quantity of radium emanation is very small, as millimicrocuries, a millimicrocurie being one-billionth of a curie. A curie is the quantity of radium emanation in equilibrium with 1 gram of radium. It seems desirable that a referee on radio activity be appointed in order that eventually a standard analytical procedure may be adopted by the association.

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No report on the determination of chloroform in drug preparations was made by the associate referee.

### REPORT ON ATOPHAN.

By WILLIAM RABAK (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee*.

At the last meeting of the association, W. O. Emery presented a paper for the writer, entitled "Report on Phenylcinchoninic Acid (Cinchophen, Atophan)"<sup>2</sup>. This paper described briefly the chemical characteristics of atophan and indicated the scope of the work that had been done preliminary to the development of a method for its estimation.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1916, 2: 116.

<sup>2</sup> *Ibid.*, 1923, 7: 32.

The method ultimately evolved was based on the acidic properties of atophan due to the presence of a free carboxyl group. The atophan that was used in the development of the method was obtained in the open market and purified by repeated recrystallization from 95 per cent alcohol. The empirical formula was verified by the preparation of the platinum salt and subsequent analysis of the same for its platinum content. The theoretical platinum content of the pure salt is 21.49 per cent; analysis of the salt prepared from the purified product showed a platinum content of 21.20 per cent. The melting point was found to be 209.5°–210.5° C., which further assured the purity of the material at hand. Using this material the following method was devised:

*Method of assay.*

Accurately weigh 1 gram of the powdered material and transfer to a 250 cc. Erlenmeyer flask by means of neutral absolute alcohol, using about 60 cc. to complete the transfer. Place on a boiling water bath and boil for 1 minute. Quickly decant through a rapid 12.5 cm. filter, catching the filtrate in a second 250 cc. Erlenmeyer flask. Repeat this operation twice, using 30 cc. of absolute alcohol each time. Rinse the flask thoroughly with boiling alcohol and finally wash filter and tip of funnel with 60 cc. of boiling absolute alcohol by means of a small wash bottle, carefully washing the upper edge of the filter paper. Add a few drops of phenolphthalein, followed by 50 cc. of 0.1N sodium hydroxide (excess), and titrate with 0.1N acid until the pink color just disappears. Subtract the number of cc. of acid used from the cc. of alkali added. Each cc. of 0.1N alkali consumed corresponds to 24.91 mg. of atophan.

In accordance with the recommendation approved by the association at the last meeting, the method was further studied, and three samples of atophan were sent to each of three collaborators. Sample No. 1 contained 70 per cent atophan and 30 per cent milk sugar; Sample No. 2 contained 45 per cent atophan and 55 per cent milk sugar; and Sample No. 3 contained 25 per cent atophan and 75 per cent milk sugar. The collaborative results obtained are embodied in the table.

COMMENTS OF COLLABORATORS.

*C. W. Harrison.*—Atophan being difficultly soluble in hot alcohol, it was not easy to wash all the alcohol soluble material through the filter paper, and 60 cc. was insufficient to complete the washing of the funnel tip and paper. The final volume of the alcoholic solution of the atophan amounted to 270 to 300 cc. It seems to me that the filtration of the alcoholic solution is unnecessary \* \* \*. You might well eliminate this filtration from the method and simply dissolve a weighed portion of your sample in hot alcohol, add an excess of standard alkali, and titrate \* \* \*.

*M. M. Woodward.*—Would not a straight titration to the color do as well? The results check rather closely with the double titration. Titrating from the color it seemed that a slight pink color persisted for some time, making the end point rather difficult of attainment.

*C. K. Glycart.*—It is my opinion that the method is satisfactory in the absence of interfering substances, because the end point obtained is fairly sharp.

TABLE 1.  
*Collaborative results.*

COLLABORATOR	SAMPLE 1.	SAMPLE 2.	SAMPLE 3.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
M. Jongeward, Fargo, N. D.	73.35 71.49	53.06 48.82	30.76 28.52
G. H. Arner, Philadelphia, Pa.	71.49 71.49	46.83 44.09	36.65 .....
C. K. Glycart, Chicago, Ill.	68.25 68.25	43.85 43.61	24.20 23.70
M. M. Woodward, Lansing, Mich.	71.16 71.29	43.89 44.01	23.14 23.34
C. W. Harrison, Baltimore, Md.	70.74 71.48	45.83 45.83	26.15 26.65
C. F. Whitney, Burlington, Vt.	73.23 73.23	46.58 47.32	27.40 27.40
Peter Valaer, Jr., Washington, D. C.	70.12 .....	44.71 ....	25.78 .....
Loren Burritt, Washington, D. C.	67.26 .. .	45.59 .. .	24.91 .....
William Rabak, Minneapolis, Minn.	70.99 69.74	44.83 44.83	25.65 25.90
Average.....	70.84	45.23	26.67

*G. H. Arner.*—The method gives good checks when sufficient care is taken to wash all the drug into the flask.

## DISCUSSION.

The titrametric method for the estimation of atophan was devised and found to be reliable as a means of assaying the U. S. P. product found on the market. For this reason it has been suggested to the U. S. P. Revision Committee as a possible addition to the present description, which contains no assay method. The method was also thought to be dependable as a means of assaying atophan tablets for their phenylcinchoninic acid content, assuming, of course, that the tablets contain no alcohol soluble interfering substances such as organic acids, etc., which would affect the titration. It is the writer's experience that atophan tablets contain only inert ingredients as excipients, there being no particular incentive for the manufacturer to add other substances, with the exception of sodium bicarbonate, which is prescribed extensively by the medical profession simultaneously with atophan in the treatment of gout and gouty conditions. Owing to this fact, it

became necessary to seek a suitable solvent for the separation of atophan from sodium bicarbonate. After a series of solubility determinations, boiling absolute alcohol was found to be the most efficacious; first, in that it would readily dissolve atophan; and second, in that it would not dissolve sodium bicarbonate.

The question of direct titration of atophan with 0.1N alkali without previous extraction with hot alcohol has been given some thought. This procedure would shorten the method and, in the absence of alkaline carbonates, no doubt would prove to be dependable.

Some study has been given to the direct titration of atophan to a pink color (phenolphthalein) after extraction, rather than away from the color as set forth in the proposed tentative method. Table 2 shows the comparative results obtained by the two different methods of titration, the same material that was sent to collaborators being used. The proposed tentative method was followed in detail up to the point where the titration is made.

TABLE 2.  
*Results obtained, using two methods of titration.*

	SAMPLE 1	SAMPLE 2.	SAMPLE 3.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Back titration with acid . . . . .	70.99	44.83	25.65
(proposed method)	69.47	44.83	25.65
Direct titration to a pink color . . . . .	69.49	45.08	25.15
	69.49	45.08	25.15

The results indicate that direct titration with 0.1N alkali is fully as accurate as the method of titration described in the proposed tentative method and has the advantage of eliminating one step in the analysis. Therefore, the method of titration is optional with the analyst.

#### RECOMMENDATIONS.

It is recommended—

- (1) That the method as given in this report be adopted as a tentative method.
- (2) That the method be further studied during the coming year with a view to eliminating imperfections.



REPORT ON QUALITATIVE AND QUANTITATIVE METHODS  
FOR THE DETERMINATION OF PYRAMIDON.

By A. W. HANSON (U. S. Food and Drug Inspection Station,  
Chicago, Ill.), *Associate Referee*.

In accordance with the recommendations of the Referee on Drugs, the methods for the determination of pyramidon, submitted last year<sup>1</sup>, were tested by co laborators.

*Description of samples.*

No. 1 contained 28.6% pyramidon, balance lactose.

No. 2 contained 54.0% pyramidon, balance lactose.

No. 3 consisted of pyramidon.

No. 4 consisted of antipyrin.

The qualitative and quantitative methods<sup>1</sup> were the same as submitted last year. The results obtained with these methods by E. O. Eaton, U. S. Food and Drug Inspection Laboratory, San Francisco, Calif.; William Rabak, U. S. Food and Drug Inspection Laboratory, Minneapolis, Minn.; E. H. Velte, H. A. Metz Laboratories, 122 Hudson St., New York City; and the associate referee are submitted as follows:

COMMENTS ON QUALITATIVE TESTS.

*E. O. Eaton.*—The color reactions on No. 3 and No. 4 appear O. K., except that Mayer's reagent gave a slight silky precipitate for pyramidon without the addition of acid.

*William Rabak.*—I found them all to be satisfactory as a means of differentiation between pyramidon and antipyrin.

*E. H. Velte:*

*Test No. 1.*—The addition of 0.1 cc. concentrated nitric acid containing some nitrous acid to 0.01 gram pyramidon in 2 cc. of water gives an immediate dark-bluish-purple color, which gradually fades out. The same test with antipyrin gives a light green color, which is permanent.

*Test No. 2.*—The addition of 0.1 cc. mercuric potassium iodide solution to 0.01 gram pyramidon in 2 cc. of water gives an immediate white flocculent precipitate. The addition of 0.1 cc. of 10% sulfuric acid causes the precipitate to become more granular with a yellow tinge and settle out, leaving a clear supernatant liquid.

*Test No. 3.*—The addition of 0.1 cc. of iodine reagent to 0.01 gram pyramidon in 2 cc. of water gives a momentary brown precipitate which changes to a clear cherry-red solution. The addition of 0.1 cc. of 10% sulfuric acid causes a slight change in color to a more purple shade. The same test with antipyrin gives a light-yellowish-brown precipitate upon addition of the iodine reagent. This gradually fades out and is brought back permanently by the addition of 0.1 cc. of 10% sulfuric acid.

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<sup>1</sup> J. Assoc. Official Agr. Chemists, 1923, 7: 29.

*Test No. 4.*—The addition of 1 cc. of ferric chloride solution to 0.01 gram of pyramidon in 2 cc. of water gives a dark purple color, which turns to a light-purplish-red color upon the addition of 0.1 cc. of 10% sulfuric acid. With antipyrin the ferric chloride solution gives a dark-brownish-red color, which does not change upon the addition of 10% sulfuric acid.

*Test No. 5.*—When 0.1 cc. of silver nitrate solution is added to 0.01 gram of pyramidon in 2 cc. of water a purple color gradually forms, metallic silver is deposited, and the solution gradually turns brown. The solution remains colorless when silver nitrate is added to antipyrin.

*Test No. 6.*—The addition of 0.1 cc. of sodium nitrite and then 0.1 cc. of 10% sulfuric acid to 0.01 gram of pyramidon in 2 cc. of water causes the gradual appearance of a purplish-blue coloration. An excess of 1 cc. each of sodium nitrite and sulfuric acid causes the solution to become gradually colorless. With antipyrin a light green color is formed. An excess of 1 cc. of sodium nitrite and sulfuric acid intensifies the color, which remains permanent. The last test has proved to be the most valuable in distinguishing pyramidon from antipyrin. The silver nitrate test is also valuable and is quite characteristic for pyramidon. The other tests are not sharp and well defined enough to admit of their general use in distinguishing the two.

#### Quantitative results.

SAMPLE No. 1.				
COLLABORATOR	BY EXTRACTION	BY TITRATION	FROM WEIGHT OF HYDROCHLORIDE	FROM CHLORIDE CONTENT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Eaton.....	29.9	..	29.36	30.3
	29.2	..	29.50	.....
Rabak.....	27.9	29.1	28.36	...
Velte.....	27.7	28.92	...	...
	27.23	28.03	...	.....
	27.32	....	..	..
Hanson.....	28.1	..	28.6	31.7
	28.4	..	28.9	33.3
SAMPLE No. 2.				
Eaton.....	55.1	.....	55.96	54.4
	55.2	..	55.4	..
Rabak.....	54.35	52.66	52.74	47.5
Velte.....	52.37	50.94	.....	..
	52.8	49.8	..	..
Hanson.....	53.8	.....	54.8	55.8
	54.3	....	54.6	58.9
SAMPLE No. 3.				
Eaton.....	100.0	97.0	103.3	105.4
	100.0	..	102.8	105.6
Rabak.....	99.25	98.15	93.80	93.39
Velte.....	98.1	96.68	...	..
	99.1	96.48	..	..
Hanson.....	99.1	97.0	100.4	105.8
	97.5	95.9	101.7	103.6

*Associate referee.*—The results on the qualitative tests are quite satisfactory. Test No. 2 for pyramidon may give a slight precipitate with Mayer's reagent before the addition of the sulfuric acid, and the addition of sulfuric acid produces a heavy precipitate. Tests 4, 5, and 6 were found to be the most satisfactory.

### COMMENTS ON QUANTITATIVE METHODS.

*E. O. Eaton.*—By determining the chloroform insoluble and air drying I found No. 1=30.1% and No. 2=54.1%.

*E. H. Velle.*—The procedure was closely adhered to. The extraction method gave the best results. The titration method came next in accuracy. The precipitation method gave uniformly high results. A more extended investigation could not be carried out owing to the smallness of the sample.

### DISCUSSION.

The table shows that the extraction method gives the most accurate results. The titration method gives low results and the precipitation method gives high results. The extraction method, while simple, does not prove that the weighed residue is all pyramidon, and a confirmatory method is desirable.

The results show that most of the excess of hydrochloric acid is removed by the procedure described, as 1 milligram of hydrochloric acid left in the residue and calculated as pyramidon would give results over 3 per cent high when 0.2 gram samples of pyramidon are taken for analysis.

It is believed from the results obtained that the pyramidon may be calculated from the weight of the hydrochloride as described in the previous report and that with certain precautions this will prove to be a more satisfactory method than the silver chloride precipitation method. The residue, consisting of pyramidon hydrochloride, can be dried at 100° C., but it is slowly volatilized if heated to 110° C. and proper care must be taken, therefore, in drying the residue of pyramidon hydrochloride.

### RECOMMENDATIONS.

It is recommended—

- (1) That qualitative methods 1, 4, 5, and 6 be adopted as tentative.
- (2) That method 1, extraction method, and method 2, hydrochloride method, be studied further by collaborators during the coming year.

No report on crude drugs was made by the associate referee.

The micromelting point apparatus, described briefly last year<sup>1</sup>, was discussed informally by J. F. Clevenger. The apparatus consists of two

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 477.

separable parts: an electric heating unit and an object unit, through which the melting point of a given substance may be observed. This arrangement permits ready separation of the units and thereby facilitates rapid cooling of the object unit for subsequent determinations. The apparatus is covered with asbestos board, glued to the metal surface with a solution of sodium silicate. This covering reduces radiation and protects the microscope. Accurate melting points are obtained by this apparatus, a detailed description of which has been published<sup>1</sup>.

## REPORT ON ALKALOIDS.

By A. R. BLISS, JR.<sup>2</sup> (College of Medicine, University of Tennessee, Memphis, Tenn.), *Associate Referee*.

The removal of the office of the Associate Referee on Alkaloids from Atlanta, Ga., to Memphis, Tenn., and the resulting additional work of completing his duties in Atlanta in a limited time, packing, unpacking, etc., forced him to postpone plans for the collaborative work of this section until some time after arrival in his new location. The work and progress to be reported at this time are consequently somewhat limited.

The work on alkaloids for 1922-23 embraced:

### A. ASSAY OF STRAMONIUM OINTMENT (*Bliss Method*).

This method, which has been published<sup>3</sup>, was submitted to six collaborators early in August 1922, but unfortunately only one collaborator reported. The results of the associate referee and M. F. Brown have been reported<sup>4</sup>. This year samples were sent to six collaborators, but not until the latter part of October. To date (November 14, 1923), no report on the method for stramonium ointment has been received; three collaborators reported their inability to carry out the work because of limited time or lack of centrifuge and mechanical shaker of sufficient size. The associate referee is planning further work on this method for 1923-24.

### B. ASSAY OF BELLADONNA OINTMENT (*G. W. Éwe Method*).

This method, which has also been published<sup>5</sup>, was submitted to five collaborators in August, 1922. One collaborator only reported. Éwe's results<sup>6</sup> and those of the associate referee and M. F. Brown<sup>7</sup> have been reported. Samples were again mailed to six collaborators in October,

<sup>1</sup> *J. Ind. Eng. Chem.*, 1924, 16: 854.

<sup>2</sup> Presented by A. G. Murray.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 1.

<sup>4</sup> *Ibid.*, 2.

<sup>5</sup> *Ibid.*, 1922, 5: 572.

<sup>6</sup> *Ibid.*, 573.

<sup>7</sup> *Ibid.*, 1923, 7: 3.

1923, but to date (November 14, 1923) no reports have been received. Three collaborators reported their inability, for one cause or another, to carry out the work at the present time. Further work on this method is planned for 1923-24.

#### RECOMMENDATIONS.

It is recommended—

(1) That the associate referee's method for the separation of quinine and strychnine<sup>1</sup> be adopted as an official method.

(2) That the method for the assay of physostigma and its preparations, as presented by G. W. Éwe<sup>2</sup>, be adopted as an official method.

(3) That the method for the assay of fluidextract of hyoscyamus, as suggested by H. C. Fuller<sup>3</sup>, be adopted as an official method.

(4) That the associate referee's method<sup>4</sup> for the assay of ointment of stramonium be adopted as a tentative method and be further studied by the collaborators.

(5) That the method for the assay of belladonna ointment, suggested by G. W. Éwe<sup>5</sup>, be adopted as a tentative method and be further studied by the collaborators.

(6) That the study of the method for the assay of belladonna liniment, suggested by G. W. Éwe<sup>5</sup>, be continued.

(7) That the study of the gravimetric and the volumetric methods for the assay of ipecac and its preparations be continued.

(8) That methods for the determination of atropine in tablets be studied.

#### METHODS FOR THE SEPARATION AND ESTIMATION OF THE PRINCIPAL CINCHONA ALKALOIDS<sup>6</sup>.

By E. O. Eaton (Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.), *Associate Referee*.

The report for 1922 on this subject had, in addition to the methods in the previous year's report, a method for separating quinidine from the three other alkaloids present.

Following certain suggestions referred to in last year's report, the method has been shortened and simplified. It has been found by preliminary trial that the use of an indicator can be dispensed with, as the

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1921, 4: 416; 1922, 5: 567.

<sup>2</sup> *Ibid.*, 1921, 4: 418; 1922, 5: 568.

<sup>3</sup> *Ibid.*, 1922, 5: 569.

<sup>4</sup> *Ibid.*, 1923, 7: 1.

<sup>5</sup> *Ibid.*, 1922, 5: 572.

<sup>6</sup> Presented by C. A. Herrmann.

limited solubility of potassium acid tartrate, formed in the presence of free hydrogen ions, insures a constant and low hydrogen ion concentration. The method for the determination of quinidine, included in the body of this report, has been made gravimetric. The identity is established by the microcrystalline structure. Mixtures of the four principal cinchona alkaloids can be separated accurately and definitely during the course of an ordinary working day.

Known samples were prepared and submitted to several collaborators for analysis. The results are given later in this report.

The presence of only one alkaloid can be readily ascertained by its specific rotation. Recrystallized anhydrous sulfates, calculated to the basis of anhydrous alkaloids, gave the following specific rotations under conditions described for the determination of quinine and cinchonidine:

Quinine	-277.4
Cinchonidine	-180.0
Cinchonine	+260.0
Quinidine	+320.0

#### DETERMINATION.

Take sufficient sample to give approximately 0.5 gram of total alkaloids and dissolve in dilute sulfuric acid. Filter if necessary, make ammoniacal, extract with chloroform to exhaustion, evaporate, dry at 110° C., and weigh.

Dissolve in 50 cc. of 0.225N sulfuric acid (225 cc. of normal sulfuric acid diluted to 1000 cc.), heat on a steam bath for 10 minutes and add cautiously, with stirring, just enough warm 5 per cent sodium hydroxide solution to make alkaline, as shown by a faint permanent precipitate. Add enough 0.225N sulfuric acid to clear the solution and then 5 cc. in excess. Add 25 cc. of saturated Rochelle salt solution to which 3 cc. of 0.225N sulfuric acid is added for each 100 cc. Remove from the steam bath, stir to start precipitation, and place in the ice box at 10°-15° C., stirring occasionally for 2 hours. Filter, and wash with 40 cc. of cold half-saturated Rochelle salt solution (dilution of above), using a small wash bottle and stirring precipitate on filter with a policeman to remove all soluble alkaloidal salts. Save combined filtrate and washings (Solution A) for other determinations. Dissolve the precipitate, consisting of the tartrates of quinine and cinchonidine, with warm dilute sulfuric acid and transfer to a separatory funnel, washing filter thoroughly to remove all alkaloids. Make solution ammoniacal and shake out to exhaustion with chloroform. Combine the chloroform extractions and run through a plug of absorbent cotton wetted with chloroform. Evaporate in a tared beaker containing a trace of sharp sand, add 5 cc. of alcohol, evaporate, dry for 3 hours at 100° C., cool, and weigh. Weight equals anhydrous quinine and cinchonidine. Dissolve without heat in 0.225N sulfuric acid exactly 0.015 gram to each cc. Transfer to a polariscope tube, filtering if necessary. (Use the longest tube possible, reducing its capacity when small quantities are worked with by inserting a straight tube of small bore, slightly shorter than a polariscope tube and fastened securely so as to center.) Use a bichromate filter and correct the reading for the zero point. Read at 20° C. Great precision is necessary for accurate determinations.

Calculate as follows:

$$(\alpha) \frac{20}{D} = \frac{10000 \times \alpha}{L \times c} \text{ (specific rotation), in which}$$

$\alpha$  = observed degrees of angular rotation;

$L$  = length of tube in mm. (i. e. 100); and

$c$  = grams of anhydrous alkaloids per 100 cc.; as prescribed in method (i. e. 1.5).

$$\text{Therefore, } (\alpha) \frac{20}{D} = 66.666 \times \alpha.$$

Substituting the value of  $(\alpha) \frac{20}{D}$  in the formula,

$$\frac{-(\alpha) \frac{20}{D} - 180}{100 \frac{20}{277.4 - 180}} = \text{per cent of quinine in the total anhydrous alkaloids, from tartrate separation.}$$

Cinchonidine is determined by difference.

Calculate these results to a percentage basis.

Place Solution A on the steam bath for 10 minutes, add 0.5 gram of potassium iodide, remove from the steam bath, and place in the ice box for 2 hours at from 10°–15° C., stirring occasionally. Filter on a weighed Gooch crucible, wash with 15 cc. of ice water, saving filtrate and washings (Solution B). Dry the precipitate of neutral quinidine hydriodide at 100° C. for 1 hour (a slight yellowing does not affect results), cool, and weigh. Weight  $\times 0.717$  equals anhydrous quinidine alkaloid. Calculate to the percentage basis.

Transfer Solution B to a separatory funnel, make ammoniacal, shake out to exhaustion with chloroform, combine chloroform extractions, and run through a plug of absorbent cotton wetted with chloroform. Evaporate cautiously in a tared beaker containing a trace of sharp sand, add 5 cc. of alcohol, evaporate, dry at 100° C. for 1 hour, cool, and weigh. Weight equals anhydrous cinchonine alkaloid. Calculate to the percentage basis.

#### Results of analysis.

SAMPLE No. 1.		RECOVERY			
		A. W. Hanson		E. O. Eaton	
		per cent		per cent	
Quinine . . . . .	0.3	96	97.3	92.4	92.4
Cinchonidine . . . . .	0.3	98.7	97.3	100.9	100.9
Cinchonine . . . . .	0.3	98.7	98.0	101.7	104.0
Quinidine . . . . .	0.3	98.7	105.3	100.7	96.1
SAMPLE No. 2					
Quinine . . . . .	0.6	99.3	96.3	98.3	98.3
Cinchonidine . . . . .	0.3	101.	102.7	98.0	98.0
Cinchonine . . . . .	0.3	104.7	108.0	107.0	99.3
Quinidine . . . . .	0.15	105.3	98.0	98.0	98.9

A. W. Hanson commented as follows: "The method does not present any special difficulty except the polarization of the quinine. A slight variation in the reading will make a considerable difference in the result. Would prefer not to use sand".

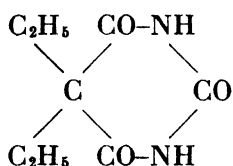
#### RECOMMENDATION.

It is recommended that these methods be made tentative, and that no further collaborative work be done.

## REPORT ON METHOD OF ANALYSIS OF BARBITAL (VERONAL) AND PHENOBARBITAL (LUMINAL).

By C. K. GLYCART (U. S. Food and Drug Inspection Station,  
Chicago, Ill.), *Associate Referee*.

Diethylbarbituric acid, or diethylmalonylurea, is the condensation product derived from diethylmalonic acid and urea, having the formula—



Molecular weight, 184.15.

This compound was introduced under the name of "veronal" in 1905 (patent expired) and also marketed as barbital. It is stated that veronal<sup>1</sup> has attained a very great clinical importance, and is now more widely used than any other synthetic hypnotic. It was formerly supposed to be practically free from toxic properties, but lately several cases of poisoning have occurred.

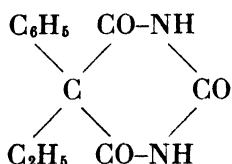
Diethylbarbituric acid is a white crystalline powder that is stable in air but sublimates when heated. It melts at 188°–189°C. It is sparingly soluble in water and chloroform, and readily soluble in alcohol and ether. It forms salts with alkalis, which are soluble in water, and is reprecipitated unchanged by acids from its alkaline solutions. Its saturated aqueous solution is acid to litmus paper.

It is stated that Deniges' reagent (mercuric sulfate), added to a saturated solution of barbital, yields a precipitate, and that Millon's reagent produces a precipitate when added to a solution of barbital acidified with nitric acid<sup>2</sup>.

### DERIVATIVES OF BARBITAL.

Luminal differs from barbital in that one ethyl group has been replaced by the phenyl group.

Formula—



<sup>1</sup> Percy May. *The Chemistry of Synthetic Drugs*. Longmans, Green and Co., 1921, p. 57

<sup>2</sup> New and Nonofficial Remedies, 1923, p. 62.

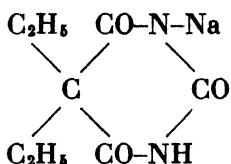


It is claimed<sup>1</sup> that the introduction of the phenyl group increases the hypnotic power of phenobarbital over barbital.

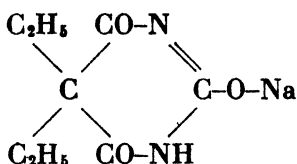
Luminal is a white odorless powder, which is almost insoluble in water and readily soluble in alcohol, ether, and in alkaline solutions. It melts at 173°–174°C.

Barbital sodium (medinal) is the monosodium salt of diethylbarbituric acid.

Formula—



or, more likely—



Luminal sodium, because of its solubility in water, may be used for hypodermic injection.

As no methods have been provided for the quantitative analysis of diethylbarbituric acid, it was thought desirable to devise a method for its estimation, and also to review the tests for its identification.

Since this compound contains no free carboxyl radical, titration failed to give a sharp end point with any indicator.

#### METHOD FOR ANALYZING BARBITAL AND ITS DERIVATIVES.

The following method, which is based on the solubility of barbital in alcohol, in the presence of chloroform, which serves for the extraction, is also applicable to the derivatives of barbital, viz., medinal, luminal and luminal-sodium.

#### REAGENTS.

(a) *Alkaline salt solution*.—To a 2% sodium hydroxide solution add salt until saturated. Filter.

(b) *Solvent*.—20 cc. of alcohol, 10 cc. of ether, and 70 cc. of chloroform. Mix.

(c) *Hydrochloric acid*.—Concentrated.

#### PREPARATION OF SAMPLE.

If in tablets, ascertain the average weight and powder a representative number for the sample.

<sup>1</sup> New and Nonofficial Remedies, 1923, p. 64.

## DETERMINATION.

Transfer 0.3 gram of the sample to a separatory funnel. Dissolve in 10 cc. of alkaline salt solution. If tablet lubricants are present, wash with 15 cc. of absolute ether and decant from top of separatory funnel into a small beaker. Add 2 cc. of concentrated hydrochloric acid to the alkaline solution, then 5 cc. of water to prevent supersaturation of salt. Extract five times, using 30, 20, 20, 10, and 10 cc. portions of the solvent. Combine the solvent in a second separatory funnel and wash with 2 cc. of water acidified with a drop of hydrochloric acid. Filter the solvent through a pledget of cotton into a small weighed beaker. Evaporate on a steam bath with the aid of an electric fan. Heat 10 minutes at 90°–100°C. Desiccate and weigh.

*General precaution:* Test for complete extraction with 10 cc. of solvent and evaporate in a separate beaker.

*Typical results of analyses of commercial samples.*

SAMPLE	ASSAY
	<i>per cent</i>
Veronal tablets.... ..	98.80
(5 grains)	98.87
Veronal . . . . .	98.67
(Imported from Switzerland)	99.30
Veronal-sodium (Medinal)... ..	99.16
(Imported)	
Luminal ... ..	99.12
(Imported from Germany)	99.73

## RECOMMENDATION.

It is recommended that samples be submitted to collaborators for further study next year.

METHODS FOR THE EXAMINATION OF SILVER PROTEINATES<sup>1</sup>.

By. E. O. EATON (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee*.

Silver proteinates are accepted by the medical profession as valuable for certain diseased conditions. As they are mildly irritant, antiseptic, and corrosive, they are preferred to the inorganic silver salts for germicidal and antiseptic purposes. They are sold extensively under various trade names and sometimes carry a statement as to composition and total silver content. They occur as a powder or granular mass and, with water, form a brown colloidal solution that is not dialyzable; they should not respond to the usual tests for silver ions. Although varying

<sup>1</sup> Presented by C. A. Herrmann.

greatly as to total silver content and as to the presence of silver ions, they fall into several general classes, based on their therapeutic effects.

The following method for total silver content is a modification of Method 3, proposed by W. L. Mitchell as associate referee in 1921<sup>1</sup>; it insures complete oxidation, also solubility of all silver present.

#### METHOD FOR TOTAL SILVER.

Place 1 gram, accurately weighed, in a 500 cc. Kjeldahl flask. Add 15 cc. of concentrated sulfuric acid and then 10 cc. of concentrated nitric acid. Boil until white fumes appear. Add more nitric acid, boil until a clear colorless solution is formed, and cool. Add 100 cc. of distilled water and boil until free of nitrogen oxides. Cool, dilute to 300 cc., add 5 cc. of nitric acid and 5 cc. of ferric alum solution, and titrate with 0.1N potassium sulfocyanate.

No. of cc. of 0.1N potassium sulfocyanate  $\times 0.010788 \times 100$  = Percentage by weight of silver.

The following procedure is used to show the presence and the percentage of silver ions.

#### QUALITATIVE TEST.

Transfer 1 gram of the preparation to a collodion dialyzing tube. (Make this by greasing the lip of a large test tube with petrolatum and add U. S. P. collodion to one-third of its depth. Pour out the collodion so as uniformly to coat the test tube and fan free of ether. Harden with cold water and carefully remove from the tube.) Add 20 cc. of distilled water to the contents of the tube and suspend it in a beaker containing 80 cc. of distilled water. Protect from the light. After 24 hours remove an aliquot portion from the beaker and test with dilute hydrochloric acid and a trace of nitric acid for silver ions.

#### QUANTITATIVE METHOD.

To 50 cc. of the above dialyzed solution, representing 0.5 gram of the original preparation, add 1 cc. of a 1 per cent gelatine solution, a few drops of dilute hydrochloric acid, and a trace of nitric acid. Compare the turbidity with a similar volume of a solution containing the same reagent and a known amount of 0.1N silver nitrate solution, and calculate to a percentage basis<sup>2</sup>.

#### ALTERNATIVE QUANTITATIVE METHOD.

To a known volume of the dialyzed solution add 1 cc. of a 1 per cent gelatine solution and follow with 1 cc. of ammonia. Add 1 cc. of a saturated aqueous hydrogen sulfide solution and make up to a definite volume. To a solution of the same reagents, add a known amount of 0.1N silver nitrate, make up to the same volume as the unknown and compare the depth of color in a colorimeter. This method presupposes the absence of metals precipitated by H<sub>2</sub>S in ammoniacal solution. The presence of a small amount of gelatin serves to hold the silver salts in colloidal solution.

No samples were submitted to collaborators. The results shown in the table were obtained by the associate referee.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 543.

<sup>2</sup> *J. Biol. Chem.*, 1919, 38: 193.

*Results of analysis.*

SAMPLE No.	TOTAL SILVER	SILVER IONS (by dialysis)
	<i>per cent</i>	<i>per cent</i>
39905, Silvol (Manufacturer's declaration "20% silver")	19.5 19.6 19.4 19.2	None.
39904 Protargol	8.9 8.7 8.9 8.9	1.0—1.0 by proposed quantitative method. 1.1—0.76 by alternative method.
39903 Argyrol	19.4 19.5	None.
Silver proteinate made in laboratory (Collargol type) <sup>1</sup>	73.8 72.7	None.

<sup>1</sup> *Year Book of the Am. Pharm. Assoc.*, 1921, 10: 340 (modified).

## RECOMMENDATION.

It is recommended that further study be given to these and any other methods available, and that collaborative work be done next year.

No report on methods for the examination of procaine was made by the associate referee.

REPORT ON METHYLENE BLUE<sup>1</sup>.

By HARRY O. MORAW (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

The iodine volumetric method<sup>2</sup> for the determination of medicinal methylene blue was adopted as a tentative method last year, and in accordance with the recommendation was studied further this year, together with moisture determinations. Samples were sent to collaborators, but the results were not received in time to include in this report.

Last year one of the collaborators, H. Wales of the Color Laboratory, Bureau of Chemistry, called attention to his belief that the U. S. Pharmacopeia is wrong in giving methylene blue with 3 molecules of water as crystal water. The writer's own experience had shown that samples of methylene blue from different manufacturers contained varying percentages of water, *i. e.*, 10.70, 13.80, 14.82, 15.40, 17.4, and 21.0, whereas the Pharmacopeial formula gives 14.44 per cent. Further investigation by Wales and Nelson<sup>3</sup> has shown that the water in

<sup>1</sup> Presented by S. Palkin.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 25.

<sup>3</sup> *J. Am. Chem. Soc.*, 1923, 45: 1657.

methylene blue is not water of crystallization. It appears, however, that since it is one of the properties of this compound to contain from 10 to 20 per cent of water in some form, there should be a maximum of water content above which the manufacturer would be required to make up the deficiency of the drug on the dry or pure color basis. It seems that the water content of 14.4 per cent, represented by the Pharmacopeial formula, is close to the average water content of different samples of the U. S. P. product examined in the Chicago laboratory. Until such time as the findings of Wales and Nelson may be corroborated and accepted as a basis for changing the Pharmacopeial formula for methylene blue, and a standard established for the moisture content, this figure may be used.

TABLE 1.  
*Results of drying methylene blue by different methods.*

METHOD OF DRYING	WEIGHT OF DISH	WEIGHT OF SAMPLE	DRYING PERIOD	LOSS	REMARKS
Electric oven 110°C.	grams 31.7927	grams 1	hours 4.5 24.5 30.5	per cent 13.07 13.52 13.63	The samples were out of the same bottle as those sent to collaborators in 1923, labeled "A.O.A.C. 1923 No. 1".
Gas oven 130°-140° C.	21.7554	2	4.5 9.0 14.0 19.0 24.0 29.0	15.60 16.10 16.92† 16.98 17.13 17.18	
Vacuum, 27 inch, temperature of boiling water.	28.9047	1	4.5 9.5	13.37 13.54	These weighings are on the same sample, heated first in vacuo 10 hours, then 18 hours in an electric oven at 110°C., then in gas oven at 135°-140° C., for about 25 hours.
Vacuum, 27 inch, temperature of boiling water.	36.0039	0.4	5.0 10.0	9.72 10.22	
Electric oven, 110° C.	36.0039	0.4	18.0	11.42	
Gas oven, 135°-140° C.*	36.0039	0.4	5.0 10.0 15.0 19.5 24.5	14.57 13.72 15.97 16.52 17.10	

\*See Table 2 for percentage of methylene blue found in sample heated to 135°C.

†After standing overnight in a sulfuric acid desiccator the loss indicated was only 16.27%.

Since the requirements of the Pharmacopeia for this product will have to be met by the manufacturers until the Pharmacopeia is changed, the assay should be adapted to the present formula. In the meantime, the referee has done some preliminary work to find out what method should be used for determining the moisture content of this product. It is understood that the usual method of determining moisture in food colors is to heat a 2 gram sample at 135° C. for one day, or until the weight is constant. This method, together with the vacuum method and heating in the electric oven at 110° C., were tried on samples. The results are shown in Table 1.

These results show that there was approximately 3.6 per cent greater loss on drying at 135° C. than by drying in vacuo or in air at 110°C. However, the charred odor of the sample, the appearance of insoluble material in the sample after heating, and the low yield of methylene blue (Table 2) when estimated in the sample after heating at 135°-140°C., show that the moisture determination should not be made at such high temperatures.

The percentage of methylene blue was determined by the iodine method in samples not heated and in samples heated by the three methods referred to above. The results in Table 2 were calculated to the dry basis, using the factor 1 cc. of 0.1N iodine = 0.006395 gram methylene blue, and the weight of the sample was calculated by deducting the moisture.

#### DISCUSSION.

Sample A in Table 2 is a product that is obviously below U. S. P. grade. It contains 6 per cent more moisture than the U. S. P. formula and appears to be an off-grade product even on the dry basis. The percentage of methylene blue in this sample, estimated by the iodine method, averages 89.7 from three determinations on the wet basis and 96.7 from two determinations on the dry basis, the difference being 7. This corresponds rather closely with the difference of 6.6 per cent between the moisture in this sample and the U. S. P. formula, namely 21 and 14.4.

This and the other samples in Table 2, whose moisture content is higher than the U. S. P. requirement have lower percentages of methylene blue on the wet than on the dry basis, and samples whose moisture content is lower than the U. S. P. requirement have higher percentages of methylene blue on the wet than on the dry basis. This is what would logically be expected, and attention is called to it to show that the iodine volumetric method is a valuable guide in showing discrepancies in the U. S. P. product.

TABLE 2.  
Loss on drying and percentage of methylene blue.

SAMPLE	LOSS ON DRYING	METHOD OF DRYING	METHYLENE BLUE BY IODINE METHOD		REMARKS
			Dry basis 1 cc. of 0.1N Iodine = 0.00639 g. in blue	U. S. P. basis 1 cc. of 0.1N Iodine = 0.007475g. in blue	
A	<i>per cent</i> 21.0	Electric oven, 110° C.	<i>per cent</i> 96.5	<i>per cent</i> .....	130 mg. weighed from a 1 gram sample heated to constant weight.
A	21.0	Not dried.	.....	88.8	130 mg. used without drying.
A1	20.9	Vacuum, 28 inch on gage, tem- perature of boiling water.	96.8	89.5	450 mg. dried to constant weight made up to 300 cc. 100 cc. aliquot = 150 mg. wet basis or 118.5 mg. dry basis.
A2	20.9	Not dried.	.....	89.7	300 mg.—200 cc.—100 cc. = 150 mg.
C	13.7	Vacuum, 28 inch, temperature of boiling water.	98.8	99.6	450 mg. made up to 300 cc. after drying. 100 cc. = 150 mg. for wet basis or 129.4 mg. dry basis.
C1	13.7	Not dried.	.....	98.6	300 mg. in 200 cc. 100 cc. = 150 mg. used.
D	13.8	Vacuum, 28 inch, temperature of boiling water.	99.9	100.6	450 mg. made up to 300 cc. after drying. 100 cc. = 150 mg. on wet basis or 129.3 mg. dry basis.
D1	13.8	Not dried.	.....	96.6	300 mg. in 300 cc. 100 cc. = 150 mg.
No. 1 A.O.A.C. 1922	10.73	Electric oven, 110° C.	97.1	101.3	450 mg. dissolved in 300 cc. after drying. 100 cc. = 150 wet basis or 133.9 mg. dry basis.
No. 1 A.O.A.C. 1922	10.70	Vacuum, 28 inch, temperature of boiling water.	97.8	99.4	450 mg. dissolved in 300 cc. after drying. 100 cc. = 150 wet basis or 133.9 mg. dry basis.
No. 1 A.O.A.C. 1922 Cryst- alline.	14.82	Vacuum, 28 inch, temperature of boiling water.	100.0	99.4	450 mg. dissolved in 300 cc. after drying. 100 cc. = 150 mg. wet basis or 127.7 mg. dry basis.
No. 1 A.O.A.C. 1923	17.18	In air 135°-140° C.		70.0	0.4 gram dissolved in 300 cc. after drying. 100 cc. = 133.33 mg.

## CONCLUSIONS.

The directions sent to collaborators for moisture determination in methylene blue called for use of the vacuum with a gage reading at least 25 inches at the temperature of boiling water. Further experience with the vacuum and the electric oven at 110° C. in air, although not indicating any material difference in the results, has led to the conclusion that when available it is easier to conduct the moisture determination in an electric oven, because the heating can be done overnight and time saved; otherwise nearly half the day may be wasted with the vacuum before it is in working order.

## RECOMMENDATIONS.

It is recommended—

- (1) That the method of drying by the electric oven at 110° C. be adopted tentatively for determining moisture in methylene blue.
- (2) That this method, together with the iodine method, be tried out by collaborators.

No report on the determination of alcohol in drugs was made by the associate referee.

G. W. Hoover: This brings us to the end of our regular program. There is one subject, which is more or less a matter of policy for the association, which I should now like to bring to your attention.

There are in the present *Methods of Analysis* certain tentative methods of analysis for preparations recognized in the U. S. Pharmacopeia; for example, fluid extract of hyoscyamus, physostigma, and powdered, solid extracts, and tincture of physostigma. The Pharmacopeia is being revised, and I understand we may expect the revision to come out within a year or a year and a half. It is reasonable to suppose that some attention has been given to any method that has not been found adequate or satisfactory in the past. The question naturally arises as to whether it is desirable to include in the *Book of Methods* additional methods of analysis for preparations that are recognized in the Pharmacopeia. Personally, I should be inclined to delete such methods from the *A. O. A. C. Methods of Analysis*. I should be glad to have the benefit of your ideas or suggestions on this subject. Is there anything involved as a general principle upon which we should take action? In view of all considerations, for the present we might do well to consider the deletion from the *Book of Methods* of methods that are duplicated. Any recommendation made by the Drug Section would be referred to the sub-committee, which committee would submit the matter to the association.



A. W. Hanson: A legal difficulty might arise if the A. O. A. C. methods should differ from the Pharmacopeial methods. Why is it not possible to have them uniform? The U. S. P. methods are being revised and so are some of the methods for the association.

G. W. Hoover: If the Pharmacopeial methods are satisfactory, of course it is not necessary for this association to devote any attention to them.

C. K. Glycart: I would make a motion that the sub-committee consider the possibility of deleting the methods under discussion and report its conclusions to the association.

A. G. Murray: The motion is seconded—that the sub-committee consider the advisability of deleting the methods from the *Book of Methods* and report to the association.

The motion was carried.

## CONTRIBUTED PAPERS.

### A STUDY OF THE ACID-SOLUBLE PHOSPHORIC ACID IN EGGS.

By LOUIS PINE (New York Station, Bureau of Chemistry, United States Department of Agriculture).

#### INTRODUCTION.

The total phosphorus pentoxide in eggs is given by Chapin and Powick<sup>1</sup> as 0.5 per cent, by Sherman<sup>2</sup> as 0.37 per cent, and by Cook<sup>3</sup> as 0.67 per cent. About 96 per cent of the total phosphorus is organically combined<sup>1</sup>. Practically all of the organic phosphorus is in the yolk. The organic compounds of the yolk containing phosphorus are proteins, chiefly vitellin, 15.7 per cent<sup>4</sup>; phospholipoids, chiefly lecithin, 11 per cent<sup>5</sup>; and glycerophosphoric acid, 1.2 per cent<sup>6</sup> of the yolk.

Chapin and Powick studied the ratio between the inorganic and the total phosphorus and its relation to the degree of deterioration of eggs. They found the inorganic phosphorus pentoxide to vary from 3.06 to 3.67 per cent of the total phosphorus pentoxide in fresh eggs, and from 7.48 to 23.78 per cent in decomposed eggs, depending upon the degree of deterioration. Cook found a decrease in lecithin in cold storage eggs.

Upon decomposition, the vitellin yields inorganic phosphoric acid<sup>7</sup>, and the phospholipoids liberate glycerophosphoric and inorganic phosphoric acids<sup>8</sup>. Therefore, the increase in the inorganic phosphoric acid alone, as shown by Chapin and Powick, would not be a complete measure of the decomposition. The increase in the total acid-soluble phosphoric acid, containing both the inorganic and glycerophosphoric acids, would be a better index of the decomposition of the organic phosphorus compounds of the yolk.

This work was undertaken to ascertain the variations in the quantity of acid-soluble phosphoric acid in eggs of good and poor quality. The white of egg was not considered in this investigation because it contains little organically combined phosphorus; therefore, the increase of acid-soluble phosphoric acid on decomposition would be negligible.

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<sup>1</sup> *J. Biol. Chem.*, 1915, 20: 97.

<sup>2</sup> *Food Products*, New York, 1916, 137.

<sup>3</sup> *U. S. Dept. Agr. Bur. Chem. Bull.* 115, 31.

<sup>4</sup> W. O. Atwater and A. P. Bryant, *U. S. Dept. Agr. Office of Experiment Station Bull.* 28 (Revised) 1906.

<sup>5</sup> A. E. Leach, *Food Inspection and Analysis*, New York, 4th ed., 1920, 271.

<sup>6</sup> J. König, *Chemie der Menschlichen Nahrungs- und Genussmittel*. Berlin, 4th ed., 1904, vol. 2, 575.

<sup>7</sup> W. D. Halliburton, *Handbook of Physiology*, Philadelphia, 11th ed., 1913, 429.

<sup>8</sup> H. Maclean, *Lecithin and Allied Substances, The Lipins*. London, 1918, 17.

## METHOD FOR THE ESTIMATION OF THE ACID-SOLUBLE PHOSPHORIC ACID IN LIQUID WHOLE EGG AND YOLK.

The three important steps taken in the determination of acid-soluble phosphoric acid in eggs are the following: the extraction, the destruction of organic matter, and the final estimation of phosphoric acid. The extraction is made by a modification of the Chapin and Powick method. The organic matter is destroyed by digestion with concentrated sulfuric and nitric acids. The phosphoric acid is determined as magnesium pyrophosphate.

## EXTRACTION.

Fifty grams of whole egg or 25 grams of yolk is weighed out in a 500 cc. Erlenmeyer flask. 200 cc. of hydrochloric acid solution, containing 1 cc. of concentrated hydrochloric acid (0.5 : 100), and 8 grams of picric acid are added. The flask is stoppered with a rubber stopper and shaken vigorously at frequent intervals, at least every 10 minutes, for 1 hour. The mixture is then filtered through a folded filter paper, 24 cm. for whole egg and 18.5 cm. for yolk. The filtration should not be allowed to proceed for more than three-quarters of an hour.

## DESTRUCTION OF ORGANIC MATTER.

125-150 cc. of the filtrate is transferred to a 500 cc. Kjeldahl flask. Four glass beads, 10 cc. of concentrated sulfuric acid, and 10 cc. of concentrated nitric acid are added. The mixture is boiled down until white fumes appear. About 2 cc. of concentrated nitric acid is added drop by drop, and the mixture is boiled again until white fumes appear. This last step is repeated four times. The mixture is boiled 10 minutes longer and then allowed to cool. About 25 cc. of water is added, and the solution is boiled until the brown fumes are driven off.

## ESTIMATION OF PHOSPHORIC ACID.

The solution, while still hot, is transferred to a 400 cc. beaker, and the flask is washed with small quantities of hot water until the volume of solution in the beaker measures about 100 cc. The phosphoric acid is then determined by the official gravimetric method<sup>1</sup>.

The total volume of solution in the extraction mixture is found by adding to the 200 cc. of hydrochloric acid solution the volume of water contained in 50 grams of whole egg or 25 grams of yolk and in 8 grams of picric acid, according to the method by Chapin and Powick.

Water in eggs is determined in vacuum at 55° C.<sup>2</sup> and in picric acid in vacuum over sulfuric acid<sup>3</sup>.

NOTE 1.—Rubber stoppers absorb picric acid. They can be cleaned by soaking in water and changing the water several times.

NOTE 2.—The quantity of filtrate collected in three-quarters of an hour depends upon the grade of filter paper used. A rapid filter paper must be used.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 1.

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull., 846.

<sup>3</sup> U. S. Pharmacopeia IX, 1916, 471.

# STUDY OF THE CHAPIN-POWICK METHOD FOR THE EXTRACTION OF ACID-SOLUBLE PHOSPHORIC ACID.

Chapin and Powick extract the acid-soluble phosphoric acid 2 hours with 200 cc. of water, 10 cc. of 2.5N hydrochloric acid, and 5-8 grams of picric acid. Their method was tested by varying the quantity of the reagents and the time of extraction.

## EFFECT OF HYDROCHLORIC ACID.

Ten cc. of 2.5N hydrochloric acid is equal to about 2 cc. of the concentrated acid. The quantity of concentrated hydrochloric acid was, therefore, varied from 1-4 cc. Each sample of whole egg was divided into four 50-gram portions, and each sample of yolk was divided into four 25-gram portions. Each portion of whole egg or yolk was extracted 2 hours with 200 cc. of hydrochloric acid solution, containing respectively 1, 2, 3, and 4 cc. of concentrated hydrochloric acid and 8 grams of picric acid. The results are given in Table 1.

TABLE 1.  
*Effect of hydrochloric acid.*

SAMPLE NO.	DESCRIPTION	HYDROCHLORIC ACID, CONCENTRATED			
		1 cc.	2 cc	3 cc.	4 cc
		Grams $Mg_3P_2O_7$ in 150 cc of Filtrate			
1	Whole egg . . .	0.0122	0.0125	0.0128	0.0126
2	Whole egg . . .	0.0140	0.0140	0.0144	0.0147
3	Whole egg . . .	0.0145	0.0149	0.0156	0.0161
4	Whole egg . . .	0.0182	0.0181	0.0185	0.0194
5	Whole egg . . .	0.0457	0.0455	0.0449	0.0459
6	Yolk . . . . .	0.0167	0.0171	0.0172	0.0183
7	Yolk . . . . .	0.0161	0.0170	0.0175	0.0177
8	Yolk . . . . .	0.0160	0.0163	0.0171	0.0175
9	Yolk . . . . .	0.0146	0.0148	0.0154	0.0157
10	Yolk . . . . .	0.0158	. . . . .	. . . . .	0.0170

## EFFECT OF PICRIC ACID.

Duplicate determinations were made, one with 5 and the other with 10 grams of picric acid. Each sample of whole egg was divided into two 50-gram portions, and each sample of yolk was divided into two 25-gram portions. Each portion of whole egg or yolk was extracted for two hours with 200 cc. of hydrochloric acid solution, containing 2 cc. of concentrated hydrochloric acid, and with 5 and 10 grams of picric acid, respectively. The results are shown in Table 2.

## EFFECT OF TIME.

Fifty-gram portions of whole egg and 25-gram portions of yolk were extracted with 200 cc. of hydrochloric acid solution, containing 2 cc. of concentrated hydrochloric acid and 8 grams of picric acid. The time of extractions was varied from one-half hour to 48 hours. The results are given in Table 3.

## DISCUSSION.

The results in Table 1 show that the quantity of acid-soluble phosphoric acid extracted from eggs is affected by the quantity of hydro-

TABLE 2.  
*Effect of picric acid.*

SAMPLE NO.	DESCRIPTION	PICRIC ACID	
		5 GRAMS	10 GRAMS
		$Mg_3P_2O_7$ in 150 cc. of Filtrate	
		gram	gram
1	Whole egg.....	0.0138	0.0141
2	Whole egg.....	0.0135	0.0137
3	Whole egg.....	0.0449	0.0450
4	Whole egg.....	0.0447	0.0446
5	Yolk.....	0.0156	0.0158
6	Yolk.....	0.0154	0.0156
7	Yolk.....	0.0164	0.0165
8	Yolk.....	0.0161	0.0158

TABLE 3.  
*Effect of time.*

SAMPLE NO.	DESCRIPTION	SHAKEN BY MACHINE		SHAKEN BY HAND EVERY 10 MINUTES				SHAKEN OCCASIONALLY	
		$\frac{1}{2}$ Hour	1 Hour	1 Hour	2 Hours	3 Hours	4 Hours	24 Hours	48 Hours
		$Mg_3P_2O_7$ in 150 cc. of Filtrate							
		gram	gram	gram	gram	gram	gram	gram	gram
1	Whole egg.....	0.0129	.....	.....	0.0138	.....	.....	0.0361	0.0684
2	Whole egg.....	0.0139	.....	.....	0.0148	.....	.....	0.0309	0.0505
3	Whole egg.....	.....	0.0131	.....	0.0136	.....	.....	.....	.....
4	Whole egg.....	.....	0.0140	.....	0.0145	.....	.....	.....	.....
5	Whole egg.....	0.0120	.....	0.0119	.....	.....	.....	.....	.....
6	Whole egg.....	0.0105	.....	0.0108	.....	.....	.....	.....	.....
7	Whole egg.....	0.0115	.....	0.0116	.....	.....	.....	.....	.....
8	Yolk.....	0.0164	.....	0.0163	.....	.....	.....	.....	.....
9	Yolk.....	0.0145	.....	0.0145	.....	.....	.....	.....	.....
10	Yolk.....	0.0153	.....	0.0156	.....	.....	.....	.....	.....
11	Yolk.....	0.0138	0.0139	.....	0.0148	0.0152	0.0164	.....	.....
12	Yolk.....	.....	.....	.....	0.0157	0.0159	0.0163	.....	.....
13	Yolk.....	.....	.....	.....	0.0145	0.0150	0.0156	.....	.....

chloric acid used in the extraction. The difference in the quantities of magnesium pyrophosphate between two successive columns is slight, but when columns 1 and 4 are compared the difference is quite marked. These results show that 1 cc. of concentrated hydrochloric acid is sufficient for the extraction of the acid-soluble phosphoric acid because increasing the amount of hydrochloric acid to 2 cc. does not affect the results appreciably.

Table 2 shows that 5 and 10 grams of picric acid give the same results. The filtration is much quicker with 10 than with 5 grams of picric acid. The maximum amount (8 grams) recommended by Chapin and Powick,

therefore, is to be preferred to the minimum (5 grams) solely on account of the effect on filtration.

Table 3 shows that one-half hour and one hour extractions give practically the same results, but if the process is continued longer, more phosphoric acid is extracted.

The higher yield of phosphoric acid obtained when more than 2 cc. of concentrated hydrochloric acid was used, or when the extraction was continued over one hour, may be due to the disintegration of organically combined phosphorus. Therefore, the Chapin and Powick method of extraction was modified as follows: The amount of hydrochloric acid was changed from 10 cc. of 2.5N (about 2.0 cc. of the concentrated acid) to 1 cc. of concentrated hydrochloric acid, and the time of extraction was decreased to 1 hour.

#### PLAN OF INVESTIGATION.

To find the normal variations of the acid-soluble phosphoric acid in whole egg and yolk of good quality, 25 samples of each were selected. These eggs were divided into two grades: strictly fresh (one day old) and market fresh. To find the maximum amount of acid-soluble phosphoric acid that exists in edible whole egg and yolk, 10 samples of each were selected from weak eggs with slightly stuck yolks which could be set free by a quick twist of the egg. These eggs are edible according to Pennington, Jenkins, and Betts<sup>1</sup>. To determine whether the increase in the acid-soluble phosphoric acid in eggs is proportional to the degree of decomposition, spots, white rots, and black rots were selected. The eggs were candled and examined out of the shell as described by Pennington, Jenkins and Betts<sup>1</sup>. Each sample of whole egg consisted of two eggs, and each sample of yolk consisted of the yolks of three eggs.

Cook states the following: "Eggs in storage for one year show a loss of weight equivalent to 10 per cent of the total weight, which loss is largely water from the white". Greenlee<sup>2</sup> reports a loss of moisture in the white and a gain in the yolk in cold storage. Since whole egg loses moisture and yolk gains moisture on standing, results on stale eggs can not be compared with those on fresh eggs, unless calculated to a dry basis.

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<sup>1</sup> U. S. Dept. Agr. Bull. 565, 1918.

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Circ. 83, 1911.

## ACID-SOLUBLE PHOSPHORIC ACID AND WATER IN WHOLE EGG.

TABLE 5.

1. Strictly fresh eggs (one day old).

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	72.94	20.9	77.2
2	75.40	23.5	95.5
3	73.00	22.9	84.8
4	72.31	21.3	76.9
5	74.18	21.4	82.8
Maximum..	75.40	23.5	95.5
Minimum..	72.31	20.9	76.9
Average....	73.57	22.0	83.4

TABLE 6.

2. Market fresh eggs.

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	73.39	22.4	84.2
2	72.89	22.9	84.5
3	73.93	24.0	92.1
4	74.69	21.8	86.1
5	73.31	23.3	87.3
6	73.06	22.1	82.0
7	73.63	23.2	88.0
8	72.59	24.1	87.9
9	73.62	20.1	76.2
10	72.34	25.5	92.2
11	71.21	23.8	82.7
12	71.69	26.5	93.6
13	73.19	19.3	72.0
14	72.43	22.7	82.3
15	71.85	25.1	89.2
16	72.68	19.9	72.8
17	73.88	21.4	81.9
18	72.98	20.5	75.9
19	71.40	21.9	76.6
20	72.63	21.2	77.5
Maximum..	74.69	26.5	93.6
Minimum..	71.21	19.3	72.0
Average....	72.87	22.6	83.3

TABLE 7.

3. *Whole egg—yolk stuck to the shell but can be set free by one quick twist of the egg.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg per 100 grams</i>
1	71.91	28.8	102.5
2	71.97	26.4	94.2
3	72.34	25.2	91.1
4	72.50	25.0	90.9
5	71.90	25.8	91.8
6	70.34	28.1	94.7
7	70.64	28.1	95.7
8	72.08	26.2	93.8
9	71.66	25.4	89.6
10	72.04	27.4	98.0
Maximum..	72.50	28.8	102.5
Minimum..	70.34	25.0	89.6
Average....	71.74	26.6	94.2

TABLE 8.

4. *Whole egg—held in cold storage 11 months.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg per 100 grams</i>
1	70.63	28.8	98.1
2	71.66	26.1	92.1
3	71.00	27.3	94.1
4	71.36	28.8	100.6
5	71.54	29.1	102.2
6	72.21	28.7	103.3
7	71.74	31.9	112.9
Maximum	72.21	31.9	112.9
Minimum..	70.63	26.1	92.1
Average ..	71.45	28.7	100.5

TABLE 9.

5. *Whole egg—yolk stuck to the shell but can be set free by several quick twists of the egg.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	68.49	30.1	95.5
2	69.07	39.9	99.9
3	69.25	35.3	114.8
4	70.09	31.8	106.3
5	67.85	34.0	105.8
6	70.35	30.0	101.2
7	68.86	33.5	107.6
8	70.52	26.2	88.9
9	72.54	28.4	103.4
10	72.56	24.2	88.2
Maximum..	72.56	35.3	114.8
Minimum..	68.49	24.2	88.2
Average....	69.96	30.4	101.2



TABLE 10.

6. *Inedible whole egg—heavy spots—yolk stuck to the shell and can not be set free by twisting the egg.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	71.30	28.8	100.0
2	70.78	28.7	98.2
3	72.22	29.4	105.8
4	70.22	36.1	121.0
5	67.78	32.6	101.2
6	69.73	29.6	97.8
7	63.60	46.4	127.5
8	63.83	41.2	113.9
9	67.66	34.6	107.0
Maximum..	72.22	46.4	127.5
Minimum...	63.60	28.7	97.8
Average....	68.57	34.2	108.0

TABLE 11.

7. *Inedible whole egg—decomposed frozen eggs—putrid odor.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	70.00	46.2	154.0
2	69.02	52.1	168.2
3	69.24	54.6	177.5
4	70.41	44.8	151.4
5	69.45	51.6	168.9
Maximum..	70.41	54.6	177.5
Minimum...	69.02	44.8	151.4
Average....	69.62	49.9	164.0

TABLE 12.

8. *Inedible whole egg—while rots.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	70.07	73.0	243.9
2	68.63	92.6	295.2
3	63.19	45.5	123.6
4	70.03	70.6	235.6
Maximum..	70.07	92.6	295.2
Minimum...	63.19	45.5	123.6
Average....	67.98	70.4	224.6

TABLE 13.  
9. *Inedible whole egg—black rots.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg per 100 grams</i>	<i>mg per 100 grams</i>
1	64.41	229.4	644.6
2	69.55	142.7	468.6
3	73.13	178.1	662.8
4	74.51	184.0	721.9
Maximum..	74.51	229.4	721.9
Minimum..	64.41	142.7	468.6
Average....	70.40	183.6	624.5

## ACID-SOLUBLE PHOSPHORIC ACID AND WATER IN YOLK.

TABLE 14.  
1. *Yolk from strictly fresh eggs (one day old).*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg per 100 grams</i>	<i>mg. per 100 grams</i>
1	47.95	51.6	99.1
2	47.41	49.4	93.9
3	47.16	50.4	95.4
4	48.06	58.1	111.9
5	47.24	54.0	102.4
Maximum.	48.06	58.1	111.9
Minimum..	47.16	49.4	93.9
Average....	47.56	52.7	100.5

TABLE 15.  
2. *Yolk from market fresh eggs.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	49.01	52.0	102.0
2	49.67	50.2	99.7
3	49.10	53.8	105.7
4	49.48	62.1	122.9
5	49.64	54.6	108.4
6	50.06	56.8	113.7
7	50.15	55.3	110.9
8	50.42	53.2	107.3
9	49.48	53.5	105.9
10	49.34	51.3	101.3
11	50.69	49.9	101.2
12	50.20	58.9	118.3
13	49.87	52.4	108.1
14	48.91	56.3	110.2
15	48.52	56.3	109.4
16	50.02	49.9	99.8
17	49.35	52.8	104.2
18	49.52	48.8	96.7
19	49.70	51.0	101.4
20	50.06	44.8	89.7
Maximum..	50.69	62.1	118.3
Minimum..	48.52	44.8	89.7
Average....	49.66	53.2	105.8

TABLE 16.

3. *Yolk—yolk stuck to the shell but can be set free by one quick twist of the egg.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	51.39	53.4	109.9
2	51.73	59.2	122.6
3	51.01	59.6	121.7
4	50.88	55.2	112.4
5	51.12	47.6	97.4
6	50.71	47.9	97.2
7	51.45	53.4	110.0
8	51.17	55.6	113.9
9	52.43	52.4	110.2
10	52.49	52.7	110.9
Maximum..	52.49	59.6	122.6
Minimum..	50.71	47.6	97.2
Average....	51.44	53.7	110.6

TABLE 17.

4. *Yolk—yolk stuck to the shell but can be set free by several quick twists of the egg.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	52.70	54.1	114.4
2	51.46	54.7	112.7
3	51.29	55.8	114.6
4	52.33	50.8	106.6
5	53.03	53.3	113.5
6	52.75	56.9	120.4
7	51.59	54.3	112.2
8	52.71	56.0	118.4
Maximum..	53.03	56.9	120.4
Minimum..	51.29	50.8	106.6
Average....	52.23	54.5	114.1

TABLE 18.

5. *Yolk from eggs held in cold storage 11 months.*

NO.	WATER	ACID-SOLUBLE $P_2O_5$	
		Original Material	Dry Basis
	<i>per cent</i>	<i>mg. per 100 grams</i>	<i>mg. per 100 grams</i>
1	52.97	66.4	141.2
2	52.83	55.5	117.7
3	53.90	57.8	125.4
4	53.89	56.7	123.0
5	54.55	57.4	126.3
6	54.30	59.2	129.5
Maximum..	54.55	66.4	141.2
Minimum..	52.83	55.6	117.7
Average....	53.74	58.8	127.2

TABLE 19.

*Summary of results on acid-soluble phosphoric acid in whole egg and yolk.*

WHOLE EGG	NUMBER OF SAMPLES	ACID-SOLUBLE- $\text{P}_2\text{O}_5$ PER 100 GRAMS DRY BASIS		
		Minimum	Maximum	Average
		<i>mg</i>	<i>mg</i>	<i>mg.</i>
1. Strictly fresh (one day old) . . . . .	5	76.9	95.5	83.4
2. Market fresh . . . . .	20	72.0	93.6	83.3
3. Yolk stuck to the shell but can be set free by one quick twist of the egg . . . . .	10	89.6	102.5	94.2
4. Held in cold storage 11 months . . . . .	7	92.1	112.9	100.5
5. Yolk stuck to the shell but can be set free by several quick twists of the egg . . . . .	10	88.2	114.8	101.2
6. Yolk stuck to the shell and can not be set free by twisting the egg . . . . .	9	97.8	127.5	108.0
7. Decomposed frozen . . . . .	5	151.4	177.5	164.0
8. White rots . . . . .	4	123.6	295.2	224.6
9. Black rots . . . . .	4	468.6	721.9	624.5
YOLK				
1. Strictly fresh (one day old) . . . . .	5	93.9	111.9	100.5
2. Market fresh . . . . .	20	89.7	118.3	105.8
3. Yolk stuck to the shell but can be set free by one quick twist of the egg . . . . .	10	97.2	122.6	110.6
4. Yolk stuck to the shell but can be set free by several quick twists of the egg . . . . .	8	106.6	120.4	114.1
5. Held in cold storage 11 months . . . . .	6	117.7	141.2	127.2

## DISCUSSION.

## WATER IN WHOLE EGG AND YOLK.

The water content of whole egg was found to vary from 75.40 per cent (Table 5) to 63.19 per cent (Table 12). One-day-old eggs gave an average of 73.57 per cent of water. Eggs that were held in cold storage for 11 months gave an average of 71.45 per cent of water. The water content of yolk was found to vary from 47.16 per cent (Table 14) to 54.55 per cent (Table 18). Yolk from eggs one day old gave an average of 47.56 per cent of water. Yolk from eggs that were held in cold storage for 11 months gave an average of 53.74 per cent of water. These results show that whole egg loses moisture and yolk gains moisture on standing. Therefore, the acid-soluble phosphoric acid must be calculated on a dry basis.

## ACID-SOLUBLE PHOSPHORIC ACID IN WHOLE EGG.

The variations in the amount of acid-soluble phosphorus pentoxide in 5 samples of strictly fresh eggs (one day old) are shown in Table 5. The minimum is 76.9, the maximum is 95.5, and the average is 83.4 milligrams per 100 grams on a dry basis. Table 6 shows that the acid-soluble phosphorus pentoxide in 20 samples of market fresh eggs varies from 72.0 to 93.6 milligrams per 100 grams on a dry basis, and the average is 83.3 milligrams.

In Table 7 are shown 10 samples of stale eggs. Fresh eggs were allowed to stand until the yolks stuck to the shells but could be set free by a quick twist of the eggs. These eggs are considered edible according to Pennington, Jenkins, and Betts<sup>1</sup>. The acid-soluble phosphorus pentoxide in these eggs varies from 89.6 to 102.5 milligrams per 100 grams on a dry basis, and the average is 94.2 milligrams. The minimum, maximum, and the average results are higher than those obtained on eggs of good quality (Table 6). These results show that after decomposition has set in, as shown by candling and physical examination out of the shell, the acid-soluble phosphoric acid is increased.

Table 8 shows 7 samples of eggs that were held in cold storage for 11 months. The condition of these eggs was as follows: the air spaces were movable, the yolks separated from the whites with great difficulty, the whites were very thin and slightly colored yellow, and the eggs had a perceptible odor. The acid-soluble phosphorus pentoxide in these eggs varies from 92.1 to 112.9 milligrams per 100 grams on a dry basis, and the average is 100.5 milligrams.

In Table 9 are shown 10 samples of eggs of a doubtful nature. These eggs can not be classed as edible or inedible. The eggs of this series were allowed to stand until the yolks stuck to the shells but could be set free by several quick twists of the eggs. As a rule, a candler does not twist an egg before the candle more than two or three times. The average candler might pass some of the eggs of this series as edible, but not all. The acid-soluble phosphorus pentoxide in these eggs varies from 88.2 to 114.8 milligrams per 100 grams on a dry basis, and the average is 101.2 milligrams. The minimum, maximum, and the average results are higher than those obtained on eggs of good quality (Table 6). The maximum and the average results are higher than those obtained on eggs the yolks of which stuck to the shells but could be set free by a single quick twist of the eggs (Table 7). The difference in age between eggs of Table 7 and Table 9 may be only a few days.

Table 10 shows 9 samples of heavy spots. The yolks of these eggs were stuck to the shells and could not be set free by twisting the eggs. This grade of eggs is considered inedible. The acid-soluble phosphorus pentoxide was found to vary from 97.8 to 127.5 milligrams per 100 grams on a dry basis, and the average is 108.0 milligrams. On further decomposition, spots are changed to white rots, and these in turn are changed to black rots. Table 12 shows 4 samples of white rots. The acid-soluble phosphorus pentoxide in these eggs varies from 123.6 to 295.2 milligrams per 100 grams on a dry basis, and the average is 224.6 milligrams. Table 13 shows 4 samples of black rots. The acid-soluble phosphorus pentoxide in these eggs varies from 468.6 to 721.9 milligrams per 100 grams on a dry basis, and the average is 624.5 milligrams.

<sup>1</sup> U. S. Dept. Agr. Bull. 565, 1918.

In Table 19 a summary of results is given on 9 grades of whole egg used in this investigation. Excluding numbers 4 and 7, this table is arranged in the order of the gradual decomposition of eggs. The results show a progressive increase in the acid-soluble phosphoric acid from an average of 83.3 milligrams of phosphorus pentoxide per 100 grams on a dry basis in fresh eggs to an average of 624.5 milligrams in black rots.

Chapin and Powick found the inorganic phosphoric acid in fresh whole egg to vary from 59.2 to 69.7 milligrams per 100 grams on a dry basis, with an average of 65.2 milligrams. The amount of acid-soluble phosphoric acid in the same grade of eggs found in this investigation varied from 72.0 to 95.5 milligrams, with an average of 83.3 milligrams. It would appear, then, that the average amount of glycerophosphoric acid in fresh whole eggs is about 18 milligrams per 100 grams on a dry basis.

#### ACID-SOLUBLE PHOSPHORIC ACID IN YOLK.

The variations in the amount of acid-soluble phosphorus pentoxide in 5 samples of yolk from strictly fresh eggs (one day old) are shown in Table 14. The minimum is 93.9, the maximum is 111.9, and the average is 100.5 milligrams per 100 grams on a dry basis. Table 15 shows that the acid-soluble phosphorus pentoxide in 20 samples of yolk from market fresh eggs varies from 89.7 to 118.3 milligrams per 100 grams on a dry basis, and the average is 105.8 milligrams.

Table 16 shows 10 samples of yolk from eggs, the yolks of which stuck to the shells but could be set free by a quick twist of the eggs. These eggs were of the same grade as those described in Table 7. The acid-soluble phosphorus pentoxide was found to vary from 97.2 to 122.6 milligrams per 100 grams on a dry basis, and the average is 110.6 milligrams. The minimum and the average results are higher than those obtained on yolk from eggs of good quality (Table 15), but the maximum is the same.

In Table 17 are shown 8 samples of yolk from eggs of the same grade as those described in Table 9. The eggs of this series were allowed to stand until the yolks stuck to the shells but could be set free by several quick twists of the eggs. The acid-soluble phosphorus pentoxide in these yolks varies from 106.6 to 120.4 milligrams per 100 grams on a dry basis, and the average is 114.1 milligrams. The minimum and the average results are higher than those obtained on yolks from eggs of good quality (Table 15), but the maximum is lower.

Table 18 shows 6 samples of yolk from eggs which were held in cold storage for 11 months. These eggs were of the same quality as those described in the discussion of Table 8. The acid-soluble phosphorus pentoxide was found to vary from 117.7 to 141.2 milligrams per 100

grams on a dry basis, and the average is 127.2 milligrams. In these yolks the minimum, maximum, and the average results are higher than those obtained on yolk from eggs of good quality (Table 15).

#### SUMMARY AND CONCLUSIONS.

The extraction procedure used by Chapin and Powick in their method for the determination of the inorganic phosphoric acid content of food products was subjected to a critical study for the purpose of ascertaining the optimum conditions for the determination of the acid-soluble phosphoric acid content of eggs.

A method that is believed to give inorganic phosphoric acid plus glycerophosphoric acid was evolved for the determination of acid-soluble phosphoric acid in eggs.

Analyses of a large number of eggs by this method failed to reveal striking differences in the acid-soluble phosphoric acid content of edible eggs of different qualities, though in general the edible eggs of poorer quality contained slightly more of this constituent, on the average, than did the fresh eggs. However, by this means it was possible to differentiate readily between edible eggs, on the one hand, and eggs which by physical examination in and out of the shell were considered to be in an advanced stage of decomposition.

#### THE SIGNIFICANCE OF UREA IN SHARK MEAL.

By D. B. DILL (Food and Drug Inspection Station, Seattle, Wash.)<sup>1</sup>.

Staedeler and Frerichs<sup>2</sup>, in 1859, reported that the flesh of selachians (including the sharks, skates and rays) contains urea. Recently Benson<sup>3</sup> has shown that the flesh of a shark, commonly called the dogfish, contains 0.5 to 0.6 per cent of urea nitrogen, on the fresh basis, while the flesh of a skate contains slightly more.

Flesh of sharks and of other selachians has limited use as food for man. Canning attempts have failed for the reason that ammonia is liberated through urea hydrolysis at the high temperature and pressure within the sealed can during sterilization. Hydrolysis of urea takes place in the flesh of some species during shipping and storing with consequent ammonia formation<sup>3</sup>.

There have been many attempts, sometimes encouraged by Government subsidies, to utilize sharks for other purposes. These efforts have

<sup>1</sup> This investigation was carried out at the Seattle Station of the Bureau of Chemistry, U. S. Department of Agriculture, under the direction of A. W. Hansen. C. L. Alaberg offered helpful suggestions in preparing the manuscript.

<sup>2</sup> *J. Prakt. Chem.*, 1858, 73: 48.

<sup>3</sup> *Proc. Am. Soc. Biol. Chem.*, 1920, 41: 40.

consisted chiefly in manufacturing leather from their skins, oil from their livers, and either fertilizer or animal food from the rest of their body.

Fish meal, whether for fertilizer or animal food, is sold on the basis of analysis. Its most valuable constituent is nitrogen. The protein content of meal intended for animal food is ordinarily calculated from the total nitrogen content, the factor 6.25 being used. The study here reported was made for the purpose of comparing the urea and protein contents of meal made from selachians with that made from bony fishes.

The methods of analysis were chiefly those of the Association of Official Agricultural Chemists<sup>1</sup>. The modified Kjeldahl-Gunning method was used for total nitrogen. Moisture was determined at 98°-100° C. at atmospheric pressure. Ether extract was determined on the dried samples, anhydrous ethyl ether being used. Five grams were ashed at low red heat in platinum dishes. The ash was dissolved in dilute hydrochloric acid and made up to volume; phosphoric acid and potassium were determined in aliquots. Phosphoric acid was determined by the volumetric molybdate method. Potassium was determined by the Lindo-Gladding method after precipitation with ammonium oxalate, evaporation of the filtrate to dryness, and ignition to remove ammonium salts.

Urea nitrogen was determined by the Van Slyke and Cullen<sup>2</sup> method. A one-gram sample was added to 50 cc. of water in the aeration apparatus, 0.2 gram of a commercial urease preparation was added, and after 20 minutes the usual reagents were added and aeration for three hours followed. Preliminary experiments in which known quantities of urea were added to a urea-free fish meal gave a 95 to 98 per cent recovery, aerating for three hours. No difficulty was experienced with the meals in question that were of normal character. The method might be unreliable with meals made from decomposed or salted fish.

Ammonia nitrogen was determined with the same reagents and apparatus, with the omission of the urease. The urea-nitrogen results were corrected for the ammonia nitrogen found. The sample of herring meal (No. 48810) was manufactured at Baranoff, Alaska. Sardine meal A was made from heads and entrails of small sardines. Sardine meal B was made from heads and entrails of large sardines. Sardine meal C was made from whole large sardines. All of the sardine meals were manufactured in southern California.

Shark meal A was manufactured at Bellingham, Washington. Shark meals B and C represent different lots from the same factory which were analyzed by a commercial chemist. The analytical results are shown in the accompanying table.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920.

<sup>2</sup> *J. Am. Med. Assoc.*, 1914, 62: 1558.



*Composition of fish meals.*

DETERMINATION	HERRING MEAL NO. 48810	SARDINE MEAL A	SARDINE MEAL B	SARDINE MEAL C	SHARK MEAL A	SHARK MEAL B*	SHARK MEAL C*
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Moisture.....	7.99	7.09	7.67	9.83	11.41	7.07	9.23
Ether extract.....	9.28	2.33	5.57	4.39	14.16	14.02	12.10
Total N.....	11.05	8.29	9.62	9.98	11.28	12.18	12.14
N×6.25.....	69.06	51.81	60.13	62.38	70.50	76.12	75.90
Urea N.....	None	None	None	None	1.93	.....	.....
Urea.....	None	None	None	None	4.13	.....	.....
Ammonia N.....	0.05	0.07	0.04	0.07	0.31	.....	.....
Total nitrogen less urea and Ammonia N.....	11.00	8.22	9.58	9.91	9.04	.....	.....
Protein.....	68.75	51.38	59.88	61.93	56.50	.....	.....
Ash.....	9.90	23.78	20.10	14.63	9.98	9.50	9.70
P <sub>2</sub> O <sub>5</sub> .....	4.27	6.71	8.44	6.33	3.37	.....	.....
K <sub>2</sub> O.....	1.13	1.39	0.38	0.46	1.20	.....	.....

\*Commercial analyses. Urea not determined.

It is clear from these analyses that shark meal A, while apparently high in protein as shown by the usual commercial analyses, is in fact lower in protein than the other types of fish meal examined. Over 4 per cent of urea was found in the one sample of shark meal in which it was determined. The two commercial analyses of other shipments of shark meal from the same factory have even higher total nitrogen contents, which suggests that 4 per cent of urea is below rather than above average.

This shark meal, mixed with cereal products, is sold as a poultry food. It is highly prized for this purpose on account of its supposedly high protein content. Dealers have stated that shark (or dogfish) meal sometimes has as high as 15 per cent nitrogen. Unless urea nitrogen is available as a food for poultry, a fraud is thus unknowingly perpetrated on the poultryman.

It can not be assumed that urea is not utilized by poultry. The question of utilization of urea and ammonium salts by animals has been the subject of many investigations. Grafe and his co-workers, Schläpfer and Turban, investigated this question from 1912 to 1915<sup>1</sup>. They concluded that there was some utilization of both ammonium salts and urea by dogs, hogs, and men. They consider that this utilization is usually a synthesis of protein, although it may consist in part of a protein sparing action. In their experiments the usual practice was to establish the animals on a low-protein and high-carbohydrate diet, with a negative nitrogen balance. Addition to this diet of urea or ammonium salts decreased the negativity of the nitrogen balance or even made it positive. During the same period, Abderhalden and his

<sup>1</sup> *Z. physiol. Chem.*, 1912, 77:1-21; 78: 485-510; 82: 347-76; 1913, 83: 25-44; 84: 60-96; 86: 347-55; 88: 389-424; 1914, 90: 75-107; *Deut. Arch. klin. Med.*, 1915, 117: 448-61.

co-workers, Hirsch and Lampé, made similar investigations but reached different conclusions<sup>1</sup>. Abderhalden does not agree that protein synthesis takes place. While in some cases he found a favorable influence of urea on nitrogen retention, in other cases he found no influence or even an unfavorable influence.

Henriques and Andersen<sup>2</sup> injected urea and ammonium salts intravenously in goats and turkeys. Both were excreted by the kidneys quantitatively, the latter unchanged and the former either unchanged or as uric acid.

Apparently no urea feeding experiments on ruminants were performed until 1919, when Völtz<sup>3</sup> carried out urea feeding experiments with sheep with favorable results. Later he succeeded in keeping a growing lamb for eight months on a diet rich in carbohydrates, nearly devoid of protein and with an appreciable quantity of urea<sup>4</sup>. Its weight increased 40 per cent during this period. The following is typical of the various diets employed:

500 grams of hydrolyzed straw  
100 grams of potato starch  
100 grams of cane sugar  
30 grams of urea  
19.2 grams of salt mixture.

Völtz and his co-workers, Dietrich and Jantzon, later established cows on a low-nitrogen ration<sup>5</sup>. They added peanut cake to the ration of one group and an equivalent quantity of nitrogen as urea to the ration of the other. The increase in milk production was nearly as great when the supplement was urea as when it was peanut cake. Völtz concludes that bacteria in the cecum synthesize protein from amides, including urea, and that the dead bodies of the bacteria are digested in the intestine.

J. Hansen<sup>6</sup> and A. Morgen and his co-workers, Schöler, Windheuser and Ohlmer<sup>7</sup>, reached similar conclusions.

A. Schennert, W. Klein, and M. Steuber<sup>8</sup> have recently made a thorough study of the utilization of urea by sheep. Metabolism experiments were performed in which complete respiration calorimeter data were obtained. They discovered that a sheep on a low-protein ration, supplemented with urea, excreted as much as one gram of nitrogen through the skin daily. This, they believe, is the explanation of the apparent nitrogen retention from urea feeding found by earlier investigators. They conclude that protein-poor, carbohydrate-rich rations

<sup>1</sup> *Z. physiol. Chem.*, 1912, 80: 136-59, 160-74; 82: 1-20, 21-95, 84: 218-22, 1915-16, 96: 1-147.

<sup>2</sup> *Ibid.*, 1914, 92: 21-45.

<sup>3</sup> *Z. Spirulind*, 1919, 42: 223-4.

<sup>4</sup> *Biochem. Z.*, 1920, 102: 151-227.

<sup>5</sup> *Ibid.*, 1922, 130: 323-431.

<sup>6</sup> *Deut. landw. Tierzucht.*, 1922, 26: 313-5.

<sup>7</sup> *Landw. Ver.-Sta.*, 1921, 99: 1-26; 1922, 99: 359-60

<sup>8</sup> *Biochem. Z.*, 1922, 133: 137-91.

seem to be better utilized when urea is added and advance the hypothesis that urea has a stimulating action on metabolism, glandular activity, and oxidation. They do not consider that protein synthesis takes place, but that urea is excreted quantitatively through the kidneys and skin. A typical ration which they employed was the following:

500 grams of hydrolyzed straw  
200 grams of potato flakes  
30 grams of urea  
18 grams of salt mixture.

It is possibly significant that the nearly vitamine-free character of this ration and of those employed by earlier investigators is a disturbing factor. Tsuji<sup>1</sup> has shown that a dog on a vitamine-free diet maintained a positive nitrogen balance for 52 days. The nitrogen balance decreased sharply to a minimum on the 69th day, then increased rapidly to a strongly positive value on the 97th day, and remained positive until death on the 107th day.

Honcamp and co-workers, Schneller, Koudela, and Müller<sup>2</sup>, have recently reported that urea is not utilized by cows when on a low-protein, high-carbohydrate diet unless easily available carbohydrates are present. Aside from emphasizing this necessity, their conclusions are parallel to those of Völtz.

Convincing experimental evidence of the utilization of urea is therefore confined to the ruminants. Most of the vertebrates have no such organ as the capacious rumen of ruminants, in which pronounced bacterial action occurs before the food enters the chief regions of absorption and in which protein synthesis from urea apparently can take place. By inference, the ability of birds to utilize urea seems remote.

In any case such a synthesis of protein is an endothermic process. The heat absorbed must be derived from oxidation processes. Thus, even though under suitable conditions urea is utilized as a nitrogen source by ruminants, it nevertheless has a negative fuel value and there must be a corresponding increase in the non-nitrogenous constituents of the ration.

The shark meal under investigation was sold with a guarantee of 70 per cent protein. This guarantee was based on a commercial determination in which the total nitrogen was multiplied by the customary factor, 6.25. The fact that nearly one-fifth of this nitrogen was urea nitrogen was overlooked.

The discrimination between protein nitrogen and urea nitrogen is probably of no significance if the meal is to be used for fertilizer.

It is clear, however, that such a discrimination should be made if the meal is intended for animal food. It has been established that urea

<sup>1</sup> *Biochem. Z.*, 1922, **129**: 194-207.

<sup>2</sup> *Z. angew. Chem.*, 1923, **36**: 45; *Biochem. Z.*, 1923, **138**: 461-96; **143**: 111-55.

nitrogen is of less value than protein nitrogen for ruminants, and it is probably of no value for other animals. Hence, analyses of fish meal which is made wholly or in part of selachians should include urea nitrogen and ammonia nitrogen determinations. In calculating protein, the factor 6.25 should be employed only after diminishing the total nitrogen content by the sum of the urea nitrogen and the ammonia nitrogen.

## DETERMINATION OF FAT IN CACAO PRODUCTS.

By LEONARD FELDSTEIN (U. S. Food and Drug Inspection Station, Denver, Colo.).

In determining ether extract in cacao products, it has often been noticed that the cacao powder clogs the filter paper and retards the flow of ether through it. The extraction proceeds slowly, and, in some instances, almost ceases. To obviate this difficulty, the following method was devised:

Digest about 1 gram of the cacao product—powder or fine shavings—on the steam bath for one-half to three-quarters of an hour with 19 cc. of 1:1 hydrochloric acid and stir occasionally. Transfer the mixture to a Röhrig tube and, after cooling, extract with 25 cc. of washed ether. Before pouring into the Röhrig tube, wash the beaker in which the digestion was made with each portion of ether. Do not shake vigorously—merely invert tube 20–25 times. Add 25 cc. of petroleum ether and again invert tube 20–25 times. Let stand 30 minutes. If an emulsion forms, roll the tube between the hands, and the emulsion will separate. Draw off into a flask or beaker through a filter paper previously wetted with a mixture of equal parts of washed ether and petroleum ether as much of the ether fat solution as possible. Re-extract the liquid remaining in the tube, using only 15 cc. of each solvent. Draw off the ethereal solution and filter into a flask. Extract a third time, using 10 cc. of each solvent, and filter the extract into the flask. Wash the lip of the spigot, and also the funnel and filter with a few cc. of a mixture of equal parts of the two solvents. (The third extraction is absolutely necessary for good results, owing to the large quantity of ether-soluble substance usually present in cacao products.) Evaporate the combined extracts slowly on the metal part of a steam bath, and dry the fat in a boiling water oven to constant weight. Remove the fat completely with petroleum ether. Dry the residue and flask one-half hour in a boiling water oven and weigh. Calculate the percentage of fat from the difference in weight.

The following table gives results obtained on four samples, both by the method described and that of the A. O. A. C.

*Results of determination of ether extract by two methods.*

PRODUCT	A O A C METHOD	NEW METHOD
	<i>per cent</i>	<i>per cent</i>
Cocoa . . . . .	23.66	23.65
Mexican chocolate* . . . . .	18.40	18.85
Chocolate coating . . . . .	33.19	33.68
"Roof Garden" ground chocolate and cocoa . . . . .	12.75	12.80

\*The Mexican chocolate appeared to consist of chocolate, crystallized sucrose, and some ground cinnamon.

## THE DETERMINATION OF MOISTURE IN FLOUR.

By LLOYD C. MITCHELL and SAMUEL ALFEND (U. S. Food and Drug Inspection Station, St. Louis, Mo.).

## INTRODUCTORY.

The present official method of this association for the determination of moisture in flour reads:

Dry a quantity of the substance, representing about 2 grams of dry material, in a current of dry hydrogen or in vacuo at the temperature of boiling water to constant weight (approximately 5 hours). If the substance be contained in a glass vessel, the latter should not be in contact with the boiling water.

In methods for the determination of moisture in many products, attention has been called frequently to the necessity of covering the weighing dish during the period of cooling. Nothing has been mentioned, however, as to whether the dish should be left open or loosely covered during the period of drying.

It was observed by the writers that when flour was dried in vacuo in loosely covered dishes, higher and more uniform moisture values were obtained than when it was dried in open dishes (Graph 1). Since a search of the available literature did not disclose any similar observations by others, the experimental work presented in this paper was conducted.

## EXPERIMENTAL.

Twelve samples from a lot of flour were tested. Six determinations were made on each sample. Two types of dishes were used (Figures 1 and 2). The covers were inserted tightly in all dishes before their removal from the oven.

The apparatus used and the conditions of drying were the following:

Oven	E. & A. 4893 <sup>1</sup>
Dish	E. & A. 2605 <sup>1</sup>
Pressure	50 mm. mercury
Temperature	98°C.
Time of drying	5 hours
Weight of sample	2 grams

The results of these analyses are given in Table 1.

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<sup>1</sup> AA Catalog Chemical and Metallurgical Laboratory Supplies, 1920.

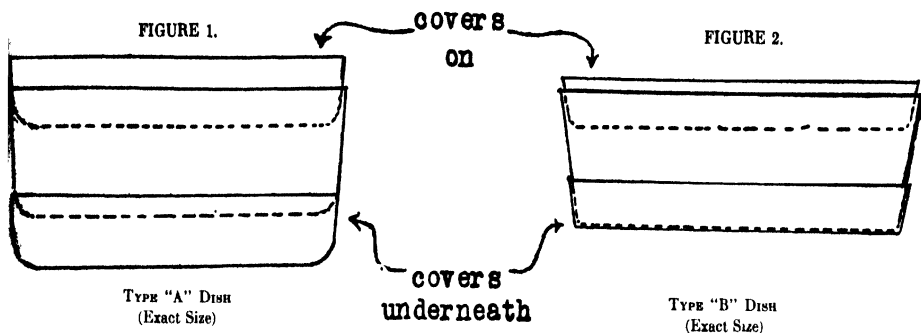
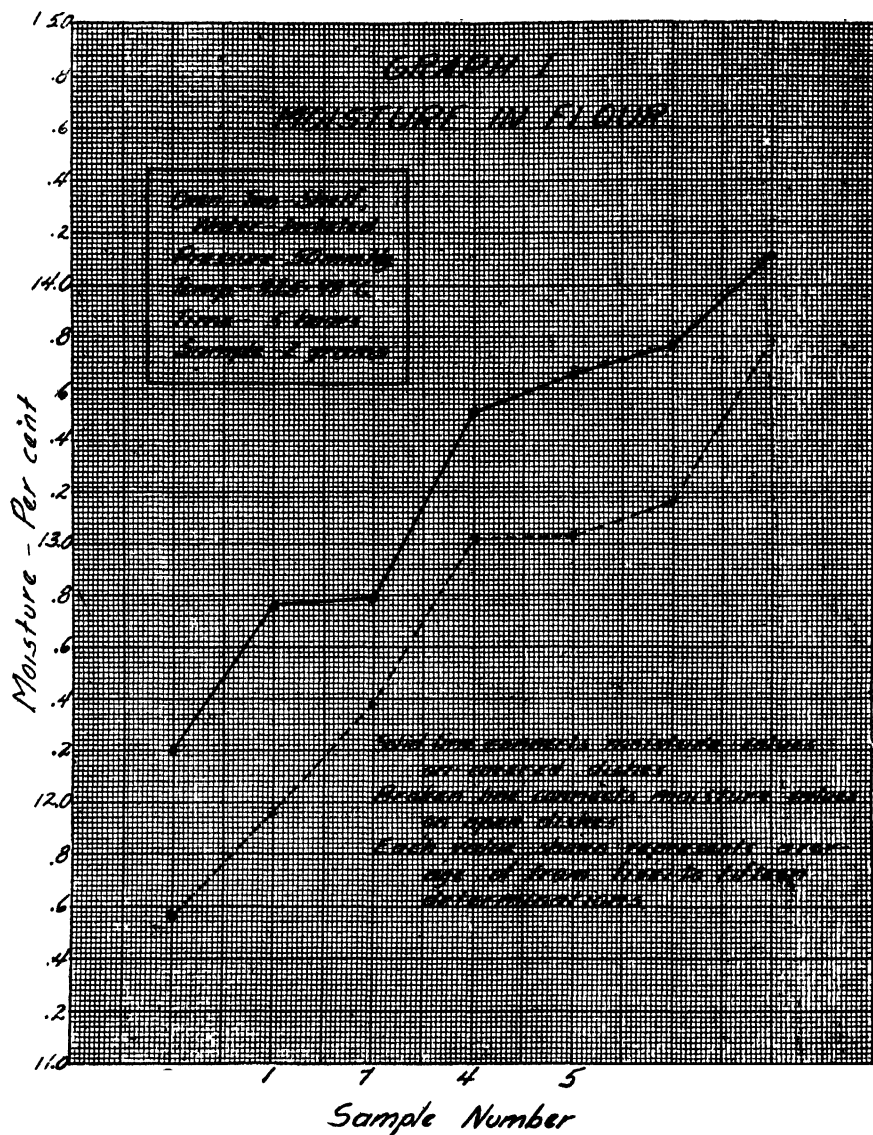


TABLE 1.  
*Moisture in flour.*

SAMPLE NO	TYPE "A" DISHES			TYPE "B" DISHES			MAXIMUM DIFFERENCE FOR EACH SAMPLE
	Lids			Lids			
	Under	On	None	Under	On	None	
FIRST ANALYST.							
2	<i>per cent</i> 13.15	<i>per cent</i> 14.04	<i>per cent</i> 13.21	<i>per cent</i> 13.35	<i>per cent</i> 14.14	<i>per cent</i> 13.37	<i>per cent</i> 0.99
4	13.32	14.17	13.39	13.35	14.10	13.38	0.85
6	13.34	14.15	13.36	13.26	14.08	13.38	0.89
8	13.39	14.08	13.42	13.59	14.21	13.63	0.82
10	13.68	14.27	13.70	13.68	14.24	13.76	0.59
12	13.73	14.25	13.90	13.90	14.15	13.94	0.52
Average	13.44	14.16	13.50	13.52	14.15	13.58	0.78
Maximum Difference	0.58	0.23	0.69	0.64	0.16	0.57	
SECOND ANALYST.							
1	13.13	13.99	13.13	13.13	13.90	13.20	0.86
3	13.58	14.19	13.56	13.41	14.16	13.49	0.78
5	13.53	14.21	13.62	13.48	14.14	13.57	0.73
7	13.51	14.12	13.56	13.65	14.22	13.55	0.71
9	13.72	14.15	13.70	13.69	14.00	13.70	0.46
11	13.71	14.02	13.77	13.75	13.90	13.75	0.31
Average	13.53	14.11	13.56	13.52	14.05	13.54	0.64
Maximum Difference	0.59	0.22	0.64	0.62	0.32	0.55	
COMBINED RESULTS.							
Average	13.48	14.14	13.53	13.52	14.10	13.56	0.72
Maximum Difference	0.60	0.28	0.77	0.77	0.34	0.74	



#### DISCUSSION.

In general, the agreement between the two analysts is very close. The outstanding feature is that the moisture values obtained by the use of covered dishes are invariably higher and check more closely than the values obtained in open dishes, the average excess being 0.60 per cent. When open dishes are used, it appears to be immaterial whether the covers are placed underneath the dishes or removed from the oven.

An examination of the table shows that in the results obtained by the use of the open dishes the moisture values in each column increase more or less gradually from the top to the bottom of the table, whereas there is no such increase in the case of the covered dishes. This is just the reverse of the order in which the dishes were covered, removed from the oven, and placed in a desiccator. The time of exposure of the uncovered dish to the air, therefore, increased gradually from the bottom to the top of the table. This would indicate that the difference between the values obtained from the covered and from the open dishes is dependent upon the time of exposure of the dishes to the air before they are covered. As it required approximately 15 minutes to release the vacuum with safety, it was physically impossible, with the available apparatus, to render nil the time of exposure of the dried sample to the air, even with a single sample. When a large number of samples were run, it required up to 10 minutes, in addition, to cover the dishes, and set them into a desiccator.

#### SUMMARY.

- (1) Higher moisture values were obtained by the use of covered dishes than by the use of open dishes.
- (2) The covered dishes gave more uniform results than the open dishes.
- (3) When covered dishes are used the position of the dishes in the oven apparently has no influence upon the moisture value obtained.

#### THE LEAD NUMBER OF VANILLA EXTRACTS.

By C. A. CLEMENS (State Food and Drug Department, Vermilion, South Dakota<sup>1</sup>).

In a recent paper<sup>2</sup> a comparison was made of the chromate and sulfate methods for the determination of lead in lead numbers of maple products. As the chromate modification was more rapid and economical, an investigation has been made as to its applicability to the determination of lead numbers in vanilla extracts.

The samples used were of commercial origin, except two extracts prepared in the laboratory by the U. S. P. method from Tahiti and Bourbon beans, respectively. In preparing the solutions for the lead determinations, the official method<sup>3</sup> was followed, as well as the method described by Wichmann<sup>4</sup>. In each case the lead was determined by precipitation as lead sulfate, as described in the official method, and also as lead chromate according to the following method:

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<sup>1</sup> Present address: Gatun, C. Z., Panama

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 350.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 198.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1921, 13: 414



#### METHOD.

To a 10 cc. aliquot of the filtrate in a 400 cc. beaker, add 2 cc. of glacial acetic acid, 25 cc. of water, and 25 cc. of approximately 0.1N potassium dichromate solution. Heat until the precipitate changes to orange-red. Transfer to a tared Gooch crucible by means of hot water and wash thoroughly. Wash with small portions of alcohol and ether and dry in a vacuum oven at the temperature of boiling water for one-half hour. Cool and weigh as  $\text{PbCrO}_4$ .  $\text{PbCrO}_4 \times 12.82 = \text{lead number}$ .

**NOTE.**—Do not put a stirring rod into the solution while boiling. Do not heat the solution before adding potassium dichromate or allow the mixture to stand after adding the potassium dichromate.

By the time the solution has come to boiling, the precipitate will have changed to an orange-red. When transferring the bulk of the solution to the Gooch crucible, use the stirring rod sparingly. If the precipitate comes in contact with the rubber policeman, wash it with hot water as soon as possible. Use the policeman vigorously in removing the precipitate from the sides of the beaker. The precipitate which adheres to the policeman can be removed by rubbing it vigorously against the sides of the beaker with an abundance of hot water. The filtrate must contain an excess of potassium dichromate; if it does not, increase the concentration or the volume of the potassium dichromate used.

Thirty-six samples were treated according to the Winton (official) method and lead determined both as lead sulfate and lead chromate. The lead numbers calculated from the sulfate determinations varied from 0.28 to 0.71, and those from the chromate determination, from 0.27 to 0.73. Only two samples with lead numbers below 0.40 were used; these were of known origin, and the low values were accounted for by incomplete extraction of the beans. Eliminating these values from the average, a value of 0.53 from the sulfate determinations and of 0.55 from the chromate determinations is shown. The differences in the lead numbers as calculated from the two modifications range from  $-0.07$  to  $+0.09$ , with an average variation of  $\pm 0.036$ . As the value expressed in a lead number is dependent on two lead determinations, a comparison of the lead actually determined would probably offer a better idea of the relation of the two methods. The amount of lead present in 10 cc. of filtrate varied from 0.0851 to 0.1108 gram. The difference between the amount of lead found by the lead sulfate precipitation and that found by the lead chromate precipitation was from  $-1.9$  to  $+3.3$  milligrams, with an average of  $\pm 1.13$  milligrams.

In making the same comparisons, using the Wichmann modification of the Winton method, thirty of the samples were used. The Wichmann lead number ranged from 0.46 to 0.98 when calculated from the lead sulfate determinations and from 0.47 to 1.06 when calculated from the chromate determinations. Only one of the weak extracts mentioned was used; when this was eliminated the minimum values were 0.57 and 0.67, respectively, and the average lead numbers, 0.78 and 0.83. The variations in the lead numbers as determined in the two ways varied from  $-0.03$  to  $+0.13$ , with an average variation of

$\pm 0.054$ . The weight of lead present in 10 cc. of filtrate in this case varied from 0.0706 to 0.1007, while the differences in results from the two methods varied from  $-2.7$  to  $+2.3$  milligrams, with an average of  $\pm 1.09$  milligrams.

On 14 blank determinations of lead, the difference between the amount of lead found by the lead sulfate and that found by the lead chromate determinations varied from  $-1.8$  to  $+2.9$  milligrams, with an average of  $\pm 1.6$  milligrams.

Wichmann found that the lead numbers determined by his modification ran about one-third higher than those determined by the official method, and on this basis calculated the minimum value for his method as 0.53. The lowest value he found was 0.55. Using commercial samples free from adulteration, as shown by analysis, and two samples prepared in the laboratory, the writer's results showed an increase in the lead number, as determined by the Wichmann modification, of about one-half over the values as determined by the official method, regardless of whether the lead number was determined as lead sulfate or as lead chromate. The percentage increase varied from 33.8 to 78.2, while the actual increase in lead number varied from 0.17 to 0.36, with an average of 0.27. Good correlation was not found between the increase and the lead number. The lowest lead number obtained by the Wichmann modification was 0.57, which agrees well with the lowest value obtained by Wichmann.

Wichmann stressed the effect of the higher temperature used in his modification. He seems to think that the better coagulation is due largely to this factor.

Experiments carried out in this laboratory seem to indicate that although this is a very important factor, dilution and the presence of alcohol also have important roles to play. Samples of extract were dealcoholized in the usual manner and heated in pressure bottles at the temperature of boiling water. No apparent coagulation took place. Another set of samples was dealcoholized and boiled over a free flame under a reflux condenser, and again the coagulation was not increased appreciably. Upon dilution of the dealcoholized extract and boiling, the coagulation was materially increased but not to the extent that was shown in the Wichmann procedure. When alcohol was added to the same solution, the precipitate granulated and filtered rapidly, yielding a clear filtrate. Satisfactory results were also obtained by diluting the original extract, adding lead acetate, and boiling under a reflux condenser. It was also noted that when extracts prepared for the Wichmann lead number determination were allowed to stand without heating, good coagulation took place in many cases, but that this was not true in every case. The Wichmann procedure gave good filtrates in every case. It is undoubtedly a great improvement over the

official method, especially if a modification can be devised so that vanillin and coumarin can be determined in the same portion or in another portion by some other than the present official method.

By using the Wichmann modification, combined with the determination of lead as chromate, the lead number determination can be completed in an hour and a half or less.

#### SUMMARY.

(1) A comparison is made of the determination of lead as sulfate and as chromate when applied to vanilla extracts.

(2) The determination of lead as chromate, as applied in the determination of lead number in vanilla extracts, is accurate, rapid, and economical.

(3) A comparison is made of the lead numbers of vanilla extracts as determined by the Wichmann modification of the Winton lead number and of those obtained by the official procedure.

(4) It is shown that the excellent coagulation of the colloidal lead precipitate, which takes place when the extract is treated according to the Wichmann modification, is due to three factors, viz., heat, dilution, and alcohol.

#### NOTE ON VANILLA EXTRACT.

By C. A. CLEMENS (State Food and Drug Department, Vermilion, South Dakota<sup>1</sup>).

Three authentic samples of vanilla extract were analyzed recently in this laboratory. The data obtained and shown in the table may be of interest.

##### *Results of analysis.*

	1	2	3
Total ash . . . . .	0.310	0.256	0.332
Insoluble ash . . . . .	0.052	0.044	0.067
Soluble ash . . . . .	0.258	0.212	0.265
Alkalinity of soluble ash . . . . .	32.5	22.5	30.5
Alkalinity of insoluble ash . . . . .	11.0	9.0	13.0
Alkalinity of total ash . . . . .	43.5	31.5	43.5
Vanillin . . . . .	0.15	0.18	0.13
Total acidity . . . . .	27.5	38.5	29.75
Lead number (a) <sup>2</sup> . . . . .	0.57	0.48	0.64
(b) See p. 79. . . . .	0.61	0.46	0.62
(c) <sup>3</sup> . . . . .	0.78	0.70	0.88
(d) See p. 79. . . . .	0.86	0.73	0.96

<sup>1</sup> Present address: Gatun, C. Z., Panama.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 198.

<sup>3</sup> *J. Ind. Eng. Chem.*, 1921, 13: 414.

(1) The extract was prepared in the laboratory from Tahiti beans by the U. S. P. method, 50 per cent alcohol being used as menstruum.

(2) The extract was prepared in the same manner as No. 1, except that Bourbon beans were used.

(3) Prepared from the same beans as No. 1, but made commercial by soaking in alcohol and extracting by the hot process.

(a) Winton lead number, the lead being determined as sulfate.

(b) Winton lead number, the lead being determined as chromate.

(c) Wichmann lead number, the lead being determined as sulfate.

(d) Wichmann lead number, the lead being determined as chromate.

These results show that the total acidity of an extract from Tahiti beans may fall below the standard suggested by Winton, Albright, and Berry<sup>1</sup> and still be a genuine extract.

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<sup>1</sup> *J. Ind. Eng. Chem.*, 1915, 7: 516.



## FIRST DAY.

### MONDAY—AFTERNOON SESSION—*Continued.*

#### REPORT ON FATS AND OILS.

By G. S. JAMIESON (Bureau of Chemistry, Washington, D. C.), *Referee.*

In accordance with the accepted recommendation (1922 report<sup>1</sup>) that further work be done on the determination of unsaponifiable matter in fats and oils, the referee, with the assistance of his collaborators, has made a comparative study of the association's official method with the method of the Committee on the Analysis of Commercial Fats and Oils of the Division of Industrial Chemists and Engineers of the American Chemical Society<sup>2</sup>.

For this investigation five samples were selected; the unsaponifiable matter ranged from about half of 1 per cent to about 4 per cent. Samples 1 to 4, inclusive, were oils, while Sample 5 was an inedible packing-house grease. Since the A. O. A. C. method for unsaponifiable residue is published in the *Book of Methods* and elsewhere, it is unnecessary to give it here. It will be recalled that it is based on making two ether extractions of the saponified fat or oil in a separatory funnel and weighing the residual unsaponifiable matter after the removal of the solvent. The second method, mentioned above and generally known as the F. A. C. procedure, is as follows:

#### F. A. C. METHOD.

*Extraction cylinder.*—The cylinder shall be glass-stoppered, graduated at 40 cc., 80 cc., and 130 cc., and of the following dimensions: Diameter about  $1\frac{3}{8}$  inches, height about 12 inches.

*Petroleum ether.*—Redistilled petroleum ether boiling under 75°C. shall be used. A blank must be made by evaporating 250 cc. with about 0.25 g. of stearine or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few milligrams.

*Determination.*—Weigh 5 g. ( $\pm 0.20$  g.) of the prepared sample into a 200 cc. Erlenmeyer flask, add 30 cc. of redistilled 95 per cent (approximately) ethyl alcohol and 5 cc. of 50 per cent aqueous potassium hydroxide, and boil the mixture for one hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 cc. mark with redistilled 95 per cent ethyl alcohol. Complete the transfer, first with warm, then with cold water, till the total volume amounts to 80 cc. Cool the cylinder and contents to room temperature and add 50 cc. of petroleum ether. Shake *vigorously* for one minute and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc. capacity. Repeat extraction at least four more times, using 50 cc. of petroleum ether each time. More extractions than five are necessary when the unsaponifiable matter runs high, say over 5 per cent, and also in some cases where it is *lower than 5 per cent, but is extracted with difficulty*. Wash the combined extracts in a separatory funnel three times with 25 cc. portions of

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 440.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1919, 11: 1161.

10 per cent alcohol, shaking vigorously each time. Transfer the petroleum ether extract to a wide-mouth tared flask or beaker, and evaporate the petroleum ether on a steam bath in an air current. Dry as in the method for moisture and volatile matter (in the vacuum oven). Any blank must be deducted from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc. petroleum ether at room temperature. Filter and wash free from the insoluble residue, if any. Evaporate and dry in the same manner as before. The committee wishes to emphasize the necessity of thorough and vigorous shaking in order to secure accurate results. The two phases must be brought into the most intimate contact possible, otherwise low and disagreeing results may be obtained.

The results reported by the collaborators are given in the table:

*Collaborative results on the determination of unsaponifiable matter.*

A. O. A. C. METHOD.

COLLABORATOR	2 ETHER EXTRACTIONS					4 ETHER EXTRACTIONS				
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2	No. 3	No. 4	No. 5
W. F. Baughman Bureau of Chemistry Washington, D. C.	0.91	1.16	2.34	0.67	3.89					
J. T. Parsons Heinz Co., Pittsburgh, Pa.	1.17	1.08	2.30	0.57	4.01	1.29	1.40 1.34	2.62	0.53	4.18 4.02
G. S. Jamieson	0.79 0.86 0.88	1.13 1.10 1.15	2.31 2.28	0.46 0.50 0.53	3.82 3.94 3.91	1.13 1.08	1.34 1.39 1.33	2.60 2.59 2.58	0.66 0.62 0.77	4.06 4.19 4.13

F. A. C. METHOD.

COLLABORATOR	5 PETROLEUM ETHER EXTRACTIONS					7 PETROLEUM ETHER EXTRACTIONS				
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2	No. 3	No. 4	No. 5
W. F. Baughman	0.76	0.99	2.05	0.44	3.67					
J. T. Parsons	0.90	0.89	2.20	0.45	3.24					
H. P. Strack Southern Cotton Oil Co. Savannah, Ga.	0.96 0.75	1.01 1.09	2.19	0.41	3.36					
H. C. Ebert Southern Cotton Oil Co. Savannah, Ga.	0.64 0.76	0.88 0.96	2.02 2.04	0.40 0.42	3.44					
E. A. Schlesser Wilson & Co., Chicago, Ill.	0.84	0.86	1.89	0.48	3.48					
G. S. Jamieson	0.68 0.78 0.73 0.77	1.03 1.09 1.03	2.09 2.15 2.08 2.12 2.17 2.18	0.41 0.44 0.56	3.42 3.42 3.41 3.65* 3.63* 3.69*			2.29 2.24		3.82 3.80 3.82

\* These higher results were due to rinsing the saponification flasks with petroleum ether, which dissolved unsaponifiable substances not previously removed by treatment with alcohol and water.

On comparing the results obtained by the A. O. A. C. method (two ether extractions) with those by the F. A. C. method (five petroleum ether extractions), it will be observed that for the most part, with the exception of Samples 3 and 5, the agreement is good. Results reported by the A. O. A. C. method for Samples 1, 2, and 4, which are higher than the average given in the table, are probably due to the fact that the unsaponifiable residues as weighed contained small amounts of soap. On the other hand, lower results were obtained for Samples 1, 2, and 4 by the F. A. C. procedure because the saponified fat was not shaken with petroleum ether long enough or with sufficient vigor. It is a well-established fact that it is absolutely necessary to shake most vigorously, as directed, in order to bring the petroleum ether in intimate contact with the unsaponifiable substances, so that they may be extracted.

In the case of the A. O. A. C. method as described, sight should not be lost of the fact that it is well known that it is impossible to extract all of the unsaponifiable substances by making only two ether extractions. It is the experience of the referee that not less than four ether extractions should be made in all cases. It will be observed in the table that a few results are reported in which four ether extractions were made. They are noticeably higher than those in which only two extractions were made. To indicate the purity of the residues weighed, the referee resaponified the residue reported under 3 as 2.58 per cent with alcoholic potash, took up in water, and made four ether extractions as before; this purified unsaponifiable matter amounted to 2.58 per cent. In the same manner the referee treated Sample 5, unsaponifiable, reported as 4.19 per cent, and obtained 4.17 per cent. This result indicates that these unsaponifiable residues, as first weighed, were of high purity. If the few results reported for four ether extractions by the A. O. A. C. method are reasonably accurate, as it is believed, then five extractions by the F. A. C. procedure are apparently inadequate for the extraction of the unsaponifiable matter. However, in the examination of edible fats and oils, reasonably accurate results can probably be obtained in the majority of instances in those cases in which the unsaponifiable matter amounts to less than 1 per cent, by either the present A. O. A. C. or the F. A. C. method. It is more than probable that when the A. O. A. C. method was studied many years ago and found satisfactory to the extent of making it an official method the investigations were confined to the analysis of edible fats and oils, which are characterized by containing small percentages of unsaponifiable substances.

It is evident, in view of the limited scope of the work of the past year, as well as the limited number of samples examined, that it will be necessary to do much more work on the comparative study of these two methods before any definite conclusions can be reached. It is going to be difficult, if not impossible, to interest collaborators who can find the



necessary time for the proposed work. This situation is largely, if not entirely, due to the fact that the A. O. A. C. procedure is not only exceedingly difficult in the manipulation, but also is very tedious and time-consuming on account of the troublesome emulsions encountered when attempting to extract the saponified samples with ether. Much more time will also be required for the proposed investigation in order to determine the actual number of ether extractions necessary to remove the unsaponifiable substances. When this information, supported by adequate analytical data, is obtained, further work must be done to determine the number of petroleum ether extractions (F. A. C. method) necessary to give results comparable with those obtained by the other method.

#### RECOMMENDATIONS.

It is recommended—

(1) That the modified Villavecchia test<sup>1</sup>, as described in the 1922 report, be adopted as an official method.

(2) That further work be done on the determination of acetyl value, including a study of the André-Cook method<sup>2</sup>.

(3) That work be continued on the determination of unsaponifiable matter or residue.

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*H. S. Bailey:* Dr. Patten has kindly consented to let me say just a word about the melting point of fats as described in the official method under the caption "Wiley Method". You that have used this method may recall that a disk of fat, 1-1.5 centimeter in diameter and weighing about 200 milligrams, is formed by allowing several drops of the melted fat to fall on a cold surface. We have been working with this method for a number of years. A mixture of oils and fats, such as are used in the manufacture of lard substitutes, does not melt like a true crystalline compound. That portion which melts most easily liquefies first, and then the remainder dissolves in this liquid rather than simply melting. To this we ascribe the great difficulty experienced in obtaining sharp melting points on such products.

In using the Wiley method in our various factory laboratories, we found it almost impossible for different chemists to get concordant results. A study of the different factors which affect the so-called melting point led finally to our devising a very simple piece of apparatus with which to cast uniform sized disks of fat. Thinking that possibly it may be of help to others I want to present it here.

We have drilled a piece of aluminum with a series of holes one centimeter in diameter. Since the plate is 2.5 millimeters thick, disks of fat

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 444.

<sup>2</sup> *J. Am. Chem. Soc.*, 1922, 44: 392.

cast in these holes meet the requirements of the present official method. In use, the aluminum plate is placed on top of a brass plate which is part of the cover of a copper box shaped much like a square shoe. The brass plate covers the toe and the rest of the box, which is about an inch higher, is open. It is filled with ice and water, and the brass plate is kept practically ice cold. This device gives the equivalent of the cold mercury surface mentioned in the official method and is much more convenient. With both plates cold, the melted fat is dropped into the holes in the aluminum plate. As soon as the fat sets, which it does almost instantly, the excess is wiped off with a warm spatula, the plate is removed, and the disks punched out of the holes. It is safest to allow the disks to stand for 3-6 hours in an ice box, as in the official method, but this is not necessary with some fats, such as vegetable shortenings.

*W. W. Skinner:* I should like to read, at this time, the following communication from Dr. David Wilbur Horn, Chairman of the Committee on Legislation of the Philadelphia Section of the American Chemical Society, addressed to the secretary of the association:

DEAR DR. SKINNER:

The enclosed data relate to a bill that passed first reading in the 1923 Legislature in Pennsylvania.

This proposed Bill sought to make it illegal to perform any laboratory procedure relating to human disease except under the supervision and upon the personal responsibility of an M. D. It authorized a committee of M. D.'s to inspect laboratories and empowered them to close such as they saw fit to close. But the Bill did not provide for any hearing or any appeal. Local legal opinion was to the effect that the power of this Bill would extend over the laboratory procedures of food analysis in every case where the procedure happened to be one that was also used in any way in the diagnosis or treatment of human disease.

In a conference with the Philadelphia physicians who were actively pushing this Bill, to which conference I was invited, I pointed out that the Bill would have a very different significance if it were reworded so that the restriction applied to the *purpose* instead of to the *procedure*. But the gentlemen would not change the wording in such a way. Accordingly some of us saw to it that this particular Bill was killed in committee in the Legislature.

The Philadelphia Section of the American Chemical Society authorizes me to submit the enclosed data to you as Secretary of the A. O. A. C., and asks action from your Society looking toward a proper surveillance of the future actions of the several State Legislatures. Such a Bill passed in *one* State would be a very useful precedent. The chemists are so outnumbered by the M. D.'s that only vigilance and prompt action when necessary can be relied upon when the Bill attempted in Pennsylvania, or any other Bill imitating it, is offered in some other State Legislature.

The data enclosed are:

(1) "Detrimental Proposed Legislation", by J. S. Hepburn, page 8, April *CATALYST*. This paper includes a copy of the Bill, and a discussion of it. On page 7 will be found the minutes of the meeting at which this matter was brought before the Section;

(2) Five mimeograph copies of the Bill;

(3) "Medical Licensure of Non-Medical Doctors", a reprint from *SCIENCE*;

(4) "Medicine and Related Arts in Chemical Laboratories", by Dr. William C. Woodward; and

(5) "Medical Licensure of Non-Medical Doctors", reprint of a reply to Dr. Woodward; both of these reprints are from *SCIENCE*.

This matter has already been brought to the attention of the A. P. A., and there is a committee at work upon it for that society. The matter is about to be brought to the attention of other national societies whose members are more or less affected by any such legislation.

*A. J. Patten*: This is a very important matter to the chemists of this country. It seems to me that some attention should be given to it by this association, and possibly it should be referred to a committee. Would you suggest the Executive Committee, Dr. Skinner? Is there any discussion of this letter?

*W. W. Skinner*: Mr. Chairman, I move that the matter be referred to the Executive Committee with power to act.

The motion was seconded and carried.

## AN IMPROVED METHOD FOR THE SEPARATION OF UNSAPONIFIABLE MATTER FROM FATS AND OILS.

By ROBERT H. KERR and D. G. SORBER (Bureau of Animal Industry, Washington, D. C.).

Methods for the determination of unsaponifiable matter are included in the methods of the Association of Official Agricultural Chemists and the standard methods of the Committee on Analysis of Commercial Fats and Oils of the American Chemical Society, and are described in texts on quantitative analysis. While the standard methods differ in the details of manipulation, all of them embody the common procedure of saponifying the fat, mixing the soap with water, and subsequently extracting the unsaponifiable matter from the aqueous soap solution with an immiscible solvent. The reverse practice of mixing the soap with the solvent and then extracting the soap from that solution with water does not appear to have been applied to the quantitative determination of unsaponifiable matter, although it is embodied in the A. O. A. C. method for the separation of cholesterol and phytosterol<sup>1</sup>. The comparatively

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 251.

prompt and effective extraction obtained by this method as contrasted with the tedious, difficult, and often incomplete extraction obtained by the standard methods led to a trial of its effectiveness for quantitative purposes. Results were satisfactory in the highest degree, being found in good agreement with those obtained both by the A. O. A. C. and the Committee on Analysis of Commercial Fats and Oils methods. A method based on this principle was then worked out in detail. This method affords a notable saving in time over the standard methods due to rapid and complete saponification, avoidance of the necessity of removing the alcohol used in saponification, and prevention of all emulsion formation in the separation. The details of the method are as follows:

Weigh 5 grams of the fat into a 150 cc. Erlenmeyer flask. Add 15 cc. of 95 per cent ethyl alcohol. Set the flask on the steam bath and heat to boiling. To another 15 cc. portion of 95 per cent ethyl alcohol add 3 cc. of concentrated potassium hydrate solution (100 grams of KOH dissolved in 100 cc. of water) and heat to boiling. When the contents of both flasks are boiling pour the potassium hydrate solution into the flask containing the fat and mix if necessary. Saponification follows forthwith, the reaction usually being vigorous. After a short period of boiling to insure complete saponification (10 minutes), remove the flask from the steam bath and allow it to cool nearly to room temperature. Add 50 cc. of ethyl ether (U. S. P.). Mix, and transfer to a separatory funnel. Wash out the flask with two successive 50 cc. portions of ether, adding these portions to the material in the separatory funnel, and mix. Add 150 cc. of distilled water, pouring it into the separatory funnel in a slow steady stream. Rotate the funnel gently to secure better contact, but do not shake. (Shaking at this stage will result in the formation of a stubborn emulsion.) Separation takes place at once and is clean and sharp. Draw off the soap solution and wash the ether layer with two successive 100 cc. portions of distilled water, still without shaking. Continue washing with successive portions of distilled water until the last portion is free from alkali and soap, as shown by testing with phenolphthalein. Transfer to a tared beaker, drive off the ether by evaporation, and dry to constant weight. Weight should be considered constant when successive dryings for one-hour periods show an additional loss of not over 0.05 per cent.

Results obtained by this method show good concordance with those obtained by the Committee on Analysis of Commercial Fats and Oils, as well as by the A. O. A. C. method when the soap was extracted four times with ether. It is recommended as a rapid, simple, and convenient method for the determination of unsaponifiable matter in fats and oils.

#### REPORT ON BAKING POWDER.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Referee*.

The collaborative work on baking powder for 1923 consisted of determining lead by an electrolytic method and carbon dioxide by a volumetric method, also in obtaining the neutralizing value of mono-calcium phosphate. The work on the determination of fluorine was directed by the associate referee, J. K. Morton, of the Bureau of Chemistry.

In order to secure a sample that would contain lead uniformly distributed throughout its mass there was prepared a special mono-calcium phosphate, in which was incorporated, at the time of its manufacture, a solution of lead chloride. This mono-calcium phosphate was then made into a baking powder and used for collaborative study. The details of the method to be followed were furnished by the associate referee. They are as follows:

## THE DETERMINATION OF LEAD IN BAKING POWDER BY ELECTROLYSIS.

(A modification of the Bryan-Corper method by J. K. Morton.)

### APPARATUS.

*Voltmeter and ammeter.*

The necessary apparatus consists of the following:

A source of direct current, together with the instruments to measure the voltage (voltmeter) and the rate of flow of the current (ammeter). The ammeter should have a capacity of 1.0 ampere and be graduated to read to 0.01 ampere. Sufficient voltage must be provided to maintain a constant current of 0.05–0.1 ampere. A storage battery of three cells and six volts capacity will provide sufficient current. A rheostat must be provided to control the rate of flow of the current. An electric stirring device is necessary to keep the electrolyte in constant motion. This is supplied by an electric motor with a glass paddle attached.

### *Electrodes.*

The anode consists of a piece of platinum foil 25 mm. square welded to a small piece of platinum wire, which is sealed into a glass tube. The wire extends into the tube about 1 cm. beyond the seal. The cathode consists of 30 cm. of No. 22 platinum wire, one end sealed into a glass tube in the same manner as the anode. The remainder of the wire is shaped into a spiral about the same width as the anode. Both anode and cathode extend vertically downward from the glass tubes in parallel planes. The glass tubes should be of sufficient diameter to admit of a column of mercury and a contact wire and long enough to admit of considerable adjustment—about 6 inches. By partly filling the tubes with mercury and inserting the wire from the circuit, contact is made with the platinum electrodes. Special care must be taken to see that the mercury does not leak around the fused-in wire.

### REAGENTS.

The following reagents are necessary:

- (a) *Hydrochloric acid* (1 + 2).
- (b) *Nitric acid* (1 + 1).
- (c) *Acetic acid* (1 + 4).
- (d) *Potassium dichromate*.—Saturated solution in water.

### PREPARATION OF SAMPLE.

Weigh 100 grams of the sample into an 800 cc. beaker. Carefully decompose the material by adding 150 cc. of hydrochloric acid (1 + 2), adding small portions at a time and stirring in well until the reaction ceases. Transfer the contents to a 600 cc. beaker and make up to a total volume of 500 cc. with water. The mixture is now ready for electrolysis.

## ELECTROLYSIS.

Arrange the electrode tubes to extend through a two-hole No. 4 rubber stopper, which will space them about 1 cm. apart. Mount them above the beaker with a clamp so they can be adjusted to any desired height. Place them well within the body of the liquid and above the blade of the stirring paddle. Adjust the beaker so that the blade of the paddle will clear the sides and bottom.

Make connections to the source of the current and start the stirring apparatus. Throw in the switch and, with the rheostat, adjust the current to show not more than 0.08, nor less than 0.05 amperes flowing. Electrolyze overnight at room temperature.

Upon completion of the electrolysis remove the pair of electrodes to a beaker of fresh water and allow the current to flow for 15 minutes. Remove the electrodes and break the current. Wash the cathode lightly with water from a wash bottle and place in a 50 cc. beaker. Remove the lead by adding 5 cc. of nitric acid and warming on the steam bath if necessary. Wash the cathode with a little more nitric acid and evaporate to dryness on the steam bath. Take up the residue with 20 cc. of acetic acid, warming if necessary. If the solution is not clear filter, and wash the filter with acetic acid. Add 2 cc. of potassium dichromate solution and allow it to stand on the steam bath for 1 hour or at room temperature overnight. The weight of lead chromate times the factor 0.641 gives the weight of lead.

NOTES: Very special care must be taken in the preparation and the weighing of the Gooch crucible.

The nitric acid residue may be a trifle brown. This is due to a little iron deposited on the cathode. The error due to this is negligible.

The nitric acid residue must be entirely free of nitric acid. In order to neutralize any nitric acid occluded in this residue, it is well to add 1 cc. of a 1 per cent solution of sodium acetate before precipitating the lead with the bichromate solution.

The potential of the current must not be allowed to drop below 4 volts.

Special care must be taken with all electrical connections to insure an uninterrupted and even flow of the electric current.

Five analysts reported on the lead determinations. Three of the five analysts secured concordant results. The agreement of their determinations is entirely satisfactory. The results of the other two analysts are so far apart that they are not to be considered. One of the analysts estimated the lead colorimetrically, instead of following the instructions to completion, which, it is thought, accounts for the low results obtained. It is not known how the other analyst obtained his results, but it is believed that the method as written is capable of producing accurate results if the directions are followed in detail. As evidence of this belief, the mono-calcium phosphate that was used to make the baking powder employed in these tests showed by this method a lead content of 94.22 and 95.51, an average of 94.87 parts per million. This baking powder was so compounded that the calcium phosphate was one-third of the finished product. On this basis the baking powder should contain 31.62 parts per million, and the average results of the three analysts were respectively 34.0, 33.95, and 32.4 parts per million. It is not probable that much closer results than these can be obtained.

TABLE 1.  
Results of electrolytic determination of lead.

ANALYST	DETERMINATIONS	PARTS PER MILLION OF LEAD
Milton H. Kemp Calumet Baking Powder Co. Chicago, Ill.	1	33.0
	2	33.0
	3	37.0
		Average 34.0
Augustus H. Fiske Rumford Chemical Works Providence, R. I.	1	10.00*
	2	13.33*
	3	15.38*
	4	16.66*
	5	16.00*
		Average 14.274
James K. Morton	1	35.25
	2	32.69
	3	32.69
	4	35.25
		Average 33.95
Ruth Buchanan Bureau of Chemistry Washington, D. C.	1	32.1
	2	32.7
		Average 32.4
J. R. Davies Calumet Chemical Co. Joliet, Ill.	1	17.9
	2	18.6
	3	19.2
	4	21.8
		Average 19.8

\* These results were determined colorimetrically.

#### A VOLUMETRIC METHOD FOR THE DETERMINATION OF CARBON DIOXIDE.

The directions for making this determination have been published in *The Journal*<sup>1</sup>. The analysts were asked to compare the volumetric method with the gravimetric method, which is now an official method<sup>2</sup>. Nine analysts reported; three of them made determinations by the gravimetric method and six by the volumetric method. The results are all in close agreement, as shown in Table 2.

One analyst, C. E. Goodrich, Bureau of Chemistry, determined both total and residual carbon dioxide by both the gravimetric and volumetric methods on seven different baking powders. His results (Table 3) show close agreement by the two methods.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 453

<sup>2</sup> *Assoc. Official Agr. Chemists Methods*, 1920, 277

TABLE 2.  
*Results of determination of carbon dioxide.*

ANALYST	BY GRAVIMETRIC METHOD		BY VOLUMETRIC METHOD
		<i>per cent</i>	<i>per cent</i>
C. J. Preston Jaques Mfg. Co. Chicago, Ill.	Average of 10 determinations.		15.005
	Maximum . . . . .		15.13
	Minimum . . . . .		14.90
G. D. Richards Jaques Mfg. Co. Chicago, Ill.	Average of 7 determinations.		15.027
	Maximum . . . . .		15.10
	Minimum . . . . .		14.95
G. D. Richards	Average of 10 determinations.		14.974
	Maximum . . . . .		15.00
	Minimum . . . . .		14.94
W. R. Collins Royal Baking Powder Co. Brooklyn, N. Y.	Average of 3 determinations.	15.033	
	Maximum . . . . .	15.06	
	Minimum . . . . .	15.02	
H. E. Hintz Royal Baking Powder Co. Brooklyn, N. Y.	Average of 3 determinations.	14.990	
	Maximum . . . . .	15.01	
	Minimum . . . . .	14.96	
Grace Vincent Royal Baking Powder Co. Brooklyn, N. Y.	Average of 7 determinations.		15.003
	Maximum . . . . .		15.10
	Minimum . . . . .		14.94
Margaret Stern Royal Baking Powder Co. Brooklyn, N. Y.	Average of 2 determinations.		15.000
	Maximum . . . . .		15.05
	Minimum . . . . .		14.95
C. H. W. Fuller American Institute of Baking 1135 Fullerton Ave. Chicago, Ill.	Average of 7 determinations.	15.164	
	Maximum . . . . .	15.30	
	Minimum . . . . .	15.07	
Ruth Buchanan	Average of 6 determinations.		15.183
	Maximum . . . . .		15.20
	Minimum . . . . .		15.10
L. H. Bailey	Average of 12 determinations.		15.096
	Maximum . . . . .		15.20
	Minimum . . . . .		15.00

Average by gravimetric method—13 determinations—15.094 per cent.

Average by volumetric method—54 determinations—15.042 per cent.

Since the volumetric method is rapid and at the same time accurate, it is believed that with slight modifications it should become an optional method.



TABLE 3.  
Results of CO<sub>2</sub> determinations made by Goodrich.

BAKING POWDER SAMPLE	BY GRAVIMETRIC METHOD		BY VOLUMETRIC METHOD	
	Total CO <sub>2</sub>	Residual CO <sub>2</sub>	Total CO <sub>2</sub>	Residual CO <sub>2</sub>
A	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	15.94	0.27	16.00	0.30
	15.84	0.38	16.00	0.30
Average	15.89	0.32	16.00	0.30
B	12.98	1.05	13.00	1.00
	13.03	0.97	13.00	0.90
Average	13.01	1.01	13.00	0.95
C	11.21	1.50	11.30	1.80
	11.33	1.63	11.20	1.75
Average	11.27	1.56	11.25	1.77
D	10.33	1.73	10.30	1.70
	10.27	1.83	10.40	1.70
Average	10.30	1.78	10.35	1.70
E	13.62	0.97	13.80	0.90
	13.50	0.86	13.95	0.85
Average	13.56	0.91	13.87	0.87
F	13.91	0.49	13.80	0.55
	13.93	0.67	13.90	0.60
Average	13.92	0.58	13.85	0.57
G	13.89	.	14.00	1.00
	13.96	.	13.90	1.00
Average	13.92	.	13.95	1.00

W. E. Stokes, of the Royal Baking Powder Company, suggests that the decomposition flask should be well shaken after the 5-minute period of standing and before the volume of gas is read, and states that a saturated sodium chloride solution has a tendency to crystallize out and creep up into the tube and also bind the stopcock. To avoid the difficulties with the sodium chloride, he proposed the use of a nearly saturated solution of sodium sulfate. To make this solution, take 644 grams of technical sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>.10 H<sub>2</sub>O) and add to 1 liter of water. When it is completely dissolved take the hydrometer reading. At 15°C. it should be 1.09 sp. gr. Make it so. Then add about 3 grams of bicarbonate of soda, about 5 cc. of methyl orange, or enough to give a good deep color, and enough sulfuric acid to make it just acid or a decided pink. Stir until

all the carbon dioxide is out. Thus the solution is at once saturated with carbon dioxide and none is absorbed from the tests. (One-half of the above proportions will be more than sufficient to fill one apparatus.)

TABLE 4.  
*Neutralizing value of mono-calcium phosphate.*

ANALYST	0.2% PHENOLPHTHALEIN SOLUTION	0.04% THYMOL BLUE SOLUTION
	<i>per cent</i>	<i>per cent</i>
W. G. Warning Provident Chemical Works St. Louis, Mo.	78.7*	79.5*
G. A. McDonald Victor Chemical Works Chicago, Ill.	76.5 77.0 77.5	78.5 79.0 79.0
M. R. Stanley Victor Chemical Works Chicago, Ill.	77.0 77.25	77.75 77.25
A. H. Allen Virginia-Carolina Chemical Co. Richmond, Va.	80.00 80.00 80.00 80.00 80.00 79.50	80.50 79.50 79.50 80.00 80.00 79.75
Augustus H. Fiske	77.00	76.5 77.0 77.0
W. C. Luckow American Institute of Baking Chicago, Ill.	76.95†	75.95†
W. R. Collins	75.25 76.15	78.80 78.35
H. E. Hintz	78.60 77.30	79.40 79.05
Grace Vincent	76.0 76.5 77.0	78.0 79.0 76.0
Ruth Buchanan	76.5 76.5 76.5 76.5	76.5 76.5 76.5 76.5
L. H. Bailey	76.5 76.5 76.0	76.5 76.5 76.5

\* Average of 3 determinations.

† Average of 8 determinations.

Average of 37 determinations . . . 76.09  
Maximum . . . . . 80.00  
Minimum . . . . . 75.25

Average of 39 determinations . . . 77.73  
Maximum . . . . . 80.50  
Minimum . . . . . 75.95

Stokes also suggested that the neck of the flask be moistened in order to make the stopper fit tightly, that the stopper be pressed in (not screwed) very hard to make the flask air-tight, and that before making an analysis on an unknown sample a test be made on a sample of known strength to insure that the apparatus is free from leaks and to lessen absorption of carbon dioxide in the sodium sulfate solution.

The referee is in accord with most of these suggestions and considers that with slight modifications the method will be found satisfactory. The referee does not believe, however, that the flask should be shaken after the 5-minute period of standing. A nearly saturated solution of either sodium chloride or sodium sulfate may be used in the leveling bulb. No objection is seen to screwing the stopper in the flask in order to make it air-tight.

#### NEUTRALIZING VALUE OF MONO-CALCIUM PHOSPHATE.

The tentative method adopted for this determination last year was modified as follows and sent to the collaborators:

Weigh 0.84 gram of mono-calcium phosphate into a 150 cc. beaker and add 25 cc. of water and 5 drops of phenolphthalein (0.2% solution). Titrate with 0.5 N sodium hydroxide to a faint pink and heat to boiling; boil 1 minute and titrate while hot to faint pink again. (Add bulk of alkali rapidly with vigorous stirring.) Total buret reading multiplied by 5 equals neutralizing strength of 100 parts of phosphate in terms of bicarbonate.

The collaborators were requested to repeat the procedure, using 0.04 per cent solution of thymol blue in place of the phenolphthalein and to report which indicator was preferred.

Eleven analysts reported on this determination. The results are all in fairly close agreement, those of one analyst being somewhat higher than the others. Some analysts preferred using 10-15 drops of the thymol blue indicator in order to secure a more distinct end point. The analysts generally prefer the phenolphthalein to the thymol blue as an indicator.

L. D. Mathias, Victor Chemical Works, calls attention to the fact that the length of time consumed in bringing the solution to the boiling point has a marked effect upon the result obtained and thinks that this point might be studied. In the results reported from that laboratory about two minutes was required to bring the solution to the boiling point.

H. F. Thomson, Provident Chemical Works, prefers a method using a more dilute solution of alkali for titrating. He suggests 0.1N sodium hydroxide and 75 cc. of water, and the use of a casserole in place of a beaker.

W. E. Stokes also prefers titrating with a more dilute solution. He suggests titrating with 0.2 N sodium hydroxide, and the use of a spot plate for the indicator, putting the indicator in the deep recesses of the spot

plate and adding thereto drops of the boiling solution. He states that the color should last one minute. The results of these suggestions are shown in Table 5.

TABLE 5.

*Neutralizing value of mono-calcium phosphate.*

(Results reported by Vincent and obtained by adding 38 cc. of 0.2 N sodium hydroxide to 0.84 gram of phosphate, boiling for 1 minute, and then finishing the titration by using the indicator on a spot plate and adding drops of the boiling solution after each addition of alkali.)

0.2% PHENOLPHTHALEIN SOLUTION	0.04% THYMOL BLUE SOLUTION
<i>per cent</i>	<i>per cent</i>
78.0	78.2
77.8	78.0
78.6	78.8
78.0	78.4
78.2	78.4
Average 78.12	78.36

To determine the correct neutralizing value of this sample of phosphate, Collins mixed it with soda, assuming its neutralizing value to be 78.5—that is, he added 1.2738 grams of phosphate to 1.000 grams of soda. The mixture had a theoretical value of 23.04 per cent carbon dioxide and had available by test 23.03 per cent, which shows 78.5 per cent to be about the correct neutralizing value.

## RECOMMENDATIONS.

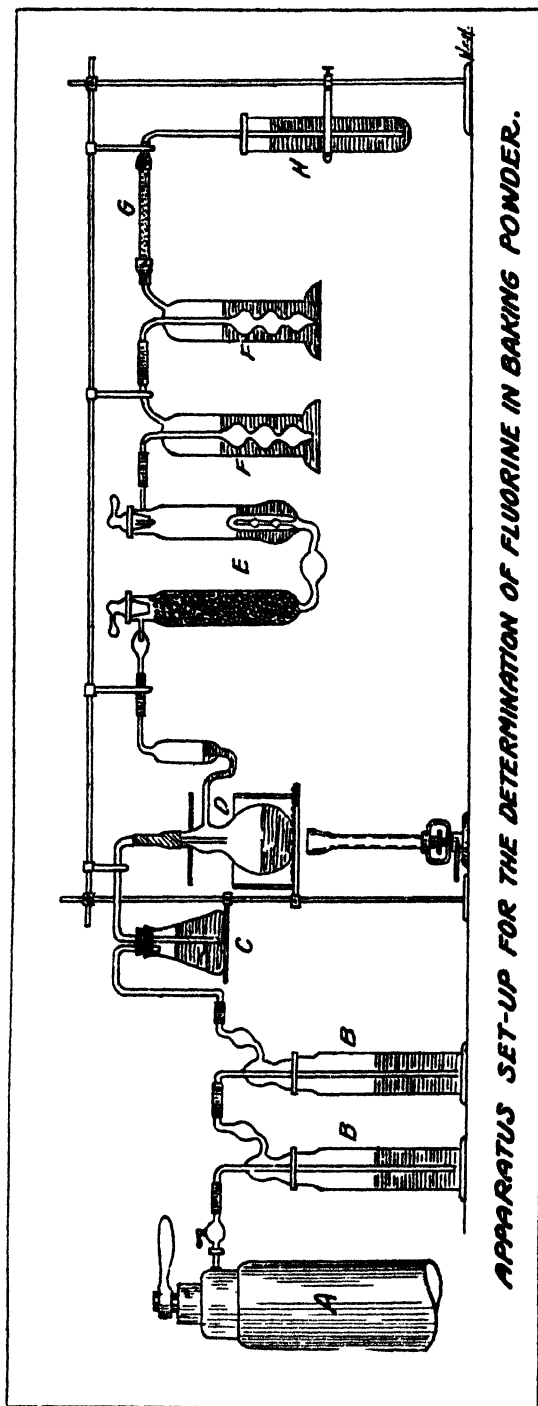
It is recommended—

(1) That the electrolytic method for the determination of lead, as outlined this year, be adopted as a tentative method.

(2) That the volumetric method for the determination of carbon dioxide, described in *The Journal*<sup>1</sup>, be modified as follows: On page 453, starting with the 12th line from the bottom, change the sentence to read, "A nearly saturated solution of sodium chloride or sodium sulfate is prepared to which a small amount of sodium bicarbonate is added"; and on page 454, under the heading, "Total Carbon Dioxide", beginning on line 13, change the sentence to read as follows: "The decomposition flask is well rotated and vigorously agitated to secure intimate contact of materials, then allowed to remain at rest for 5 minutes".

(3) That the tentative method for determining the neutralizing value of mono-calcium phosphate be changed as given in the text of this report.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 4.



- (A) A source of air, carbon dioxide, or nitrogen. Any of these mediums may be used, but the pressure must be constant.
- (B) A drying train, followed by
- (C) A 100 cc. flask from which the anhydrous sulfuric acid is introduced into the digestion flask.
- (D) A 4 ounce Pyrex distilling flask, having a small trap and reflux tube at its outlet, and into which the weighed sample is introduced.

- (E) A Schmidt tube containing glass beads in one arm and, in the other, a 10 per cent solution of silver sulfate in anhydrous sulfuric acid.
- (F) Bowen bulbs containing a saturated solution of dry chromic acid in anhydrous sulfuric acid.
- (G) A glass tube (6 x  $\frac{3}{8}$  inches) containing glass wool.
- (H) Test tube containing water into which the silicon tetrafluoride is delivered.

## REPORT ON FLUORIDES IN BAKING POWDER.

By JAMES K. MORTON (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The work of 1923 has consisted entirely of an attempt to bring about a definite procedure in the determination of fluorine in baking powder. To this end the Wagner-Ross<sup>1</sup> method has been rewritten in detail, with consideration of every factor and suggestion advanced by the collaborators of 1922.

The difficulty attending the determination of fluorine is aptly illustrated in a letter received from one of the collaborators, who, in reply to a letter asking for his assistance, made the following comment: "We are very much interested in the matter of the determination of fluorides and will be glad to collaborate on any method that has promise of success. We wish, however, to be very frank in this matter. We have had several different chemists work on the Wagner-Ross method and we have never had any success with it at all. For us to succeed with this method it will be necessary to furnish us with a very detailed description of the procedure, as with all the variations we have ourselves tried, we have not obtained positive results".

The results submitted by this collaborator are as nearly correct as the limitations of the method will permit.

A VOLATILIZATION METHOD FOR THE DETERMINATION OF FLUORINE  
IN BAKING POWDER.

(An adaptation of the Wagner-Ross Method)

The method, in general, consists in the treatment of the sample with anhydrous sulfuric acid in the presence of silica; the acid decomposes the fluorides with the liberation of silicon tetrafluoride. This gas is passed through a series of wash solutions to remove halogen acids, nitric acid, and sulfur compounds, and finally into water, with which it reacts to form hydrofluosilicic and silicic acids. The hydrofluosilicic acid is titrated with standard alkali and the fluorine content calculated.

## APPARATUS.

The apparatus is shown in the drawing.

## REAGENTS.

(a) *Copper sulfate*.—Anhydrous C. P.

(b) *Quartz flour (silica)*.—Thoroughly digest in strong hydrochloric acid, wash free of acid with water, and dry.

(c) *Silver sulfate in anhydrous sulfuric acid*.—10 per cent solution. Ignite the silver sulfate with an excess of the acid to drive off any possible volatile acids.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1917, 9: 1116

(d) *Chromic acid in anhydrous sulfuric acid*.—A saturated solution.

(e) *Glass beads and glass wool*.—Prepare by digesting in strong acid, wash, and dry.

(f) *Magnesium nitrate*.—10 per cent solution.

(g) *Potassium hydroxide*.—0.1N solution.

(h) *Sulfuric acid*.—Must be anhydrous (98.5 per cent). Prepare by boiling the ordinary 95 per cent acid in an open casserole to about two-thirds of its original volume. Pour hot into a Pyrex Erlenmeyer flask and cool under a calcium chloride tube.

NOTE.—The absence of water in the apparatus or materials used is essential to a maximum recovery of fluorine.

#### DETAILS OF THE PROCEDURE.

##### *Preparation of the ash.*

Take 20 grams of baking powder in an 11 cm. porcelain evaporating dish. Place it in a muffle furnace at a low heat and allow the organic matter to burn off as much as possible without allowing the furnace to come to the point where any redness is discernible, about 450°C. The resulting ash will be quite dark. Remove the dish and allow to cool. Powder the material and add 5 cc. of a 10 per cent solution of magnesium nitrate. Be sure that the material is thoroughly saturated. Drive off the excess of moisture on the steam bath and reheat in the muffle as before. Repeat this operation once. Keep the material in a desiccator until used.

#### DETERMINATION.

Place the ash, together with 1 gram of quartz flour and 5 grams of anhydrous copper sulfate, in the digestion flask and thoroughly mix. Replace the digestion flask in its position in the train. Pour 75 cc. of the anhydrous sulfuric acid into the 100 cc. flask (C) and replace it in its position. Add 15 cc. of water to the test tube.

Start the air very slowly and allow the pressure to force the sulfuric acid in flask C over into the digestion flask. By manipulation allow the first portion of the acid to flow into the trap and form a seal.

Carefully regulate the flow of air to give just enough headway to prevent any back pressure. Apply heat to the digestion flask slowly, bringing the temperature to about 300°C. (below the boiling point) and maintain this temperature for 2 hours. Shake the flask frequently during the heating. Before removing the flame bring to boiling and boil for 5 minutes. Remove the flame and allow the air to pass for 30 minutes to wash out any silicon tetrafluoride remaining in the system. Disconnect the delivery tube and test tube and transfer the solution to a 500 cc. Erlenmeyer flask. Dilute with water to about 250 cc., bring to boiling, and boil gently for 10 minutes; cool to 50°C. and titrate the hydrofluosilicic acid with 0.1N alkali, using phenolphthalein as an indicator.

One cc. of 0.1N alkali = 0.0019 gram of fluorine.

After titrating make the solution acid with hydrochloric acid and test for sulfates with barium chloride. Carefully estimate the amount, if any, and also the correction.

#### NOTES.

In assembling the apparatus it is well to keep the volume of the wash solution as low as possible. Ten cc. of the silver sulfate solution in the Schmidts tube, 25 cc. of the chromic-sulfuric acid solution in each of the Bowen bulbs, and 15 cc. of water in the test tube are sufficient. If the carbon in the sample has been destroyed and the rate of flow of the air

has not been too fast, there should be little, if any, sulfate in the absorption tube. The rubber connections on the apparatus should be made carefully.

A good grade of thick wall tubing should be used, and the ends of the glass apparatus should be brought close together. An asbestos shield should be placed above the bulb of the digestion flask to protect the rubber connection.

The wash solutions can be used a number of times, but they should be renewed when they show indications of becoming exhausted. This condition is usually indicated by a perceptible greenness in the first Bowen bulb and the presence of sulfate in the absorption tube.

When not in use the apparatus should be closed to prevent the absorption of the moisture from the air.

Where the air pressure is not fairly constant it is recommended that carbon dioxide be used.

Before making a determination on baking powder the material should be tested as to its alkalinity. This can readily be done by taking 5 grams of the sample, digesting in 50 cc. of water, filtering, and adding a drop of phenolphthalein to the filtrate. If it is found to be acid, add just enough sodium bicarbonate to assure an alkaline reaction.

#### DESCRIPTION OF SAMPLES.

*Sample No. 1:* A commercial grade of baking powder, prepared for the use of the referee and sent out in the original package. It contains the small amount of fluorine normally found in a phosphate baking powder. A series of determinations was made on this material to ascertain as nearly as possible its fluorine content. Assuming the average of these results, 0.0169 per cent, to be 90 per cent recovery, the fluorine content would be 0.0187 per cent.

*Sample No. 2:* The same baking powder as used in Sample No. 1 to which has been added 0.0822 per cent of fluorine as calcium fluoride, giving it a fluorine content of 0.1009 per cent.

In two instances the collaborators, in order to familiarize themselves with the method, conducted a series of controls on pure samples of calcium and sodium fluoride. As the results may be of interest they are given in Table 2. The samples of sodium fluoride are not identical, but there is no reason to believe that the fluorine content varies to any extent.

L. D. Mathias, of the Victor Chemical Company, sent in an excellent report. Two or three minor suggestions were offered, and, so far as possible, these are incorporated in the method presented in this report.

The results of three of the collaborators are in satisfactory agreement on both samples. Of the other results submitted a fair proportion is very good.



TABLE 1.  
Results of determination of fluorine in baking powder.

COLLABORATOR	FLUORINE FOUND	
	Sample No. 1 (Fluorine calculated— 0.0187 %)	Sample No. 2 (Fluorine calculated— 0.1008 %)
	<i>per cent</i>	<i>per cent</i>
Milton H. Kemp Calumet Baking Powder Co., Chicago, Ill.	0.02	0.092
	0.019	0.092
	0.022	0.088
	0.019	0.091
	Average	0.02
J. L. Howerton Federal Phosphorus Co., Anniston, Ala.	0.027	0.106
	0.023	0.105
	Average	0.025
A. B. Higgins Rumford Chemical Co., Providence, R. I.	0.016	0.0988
	0.052	0.0979
	Average	0.034
N. D. Harvey Rumford Chemical Co., Providence, R. I.	0.033	0.100
	0.027	
	Average	0.030
A. H. Allen Virginia-Carolina Chemical Co., Richmond, Va.	0.040	0.060
	0.037	0.054
	0.038	0.057
	Average	0.0383
James K. Morton	0.0173	0.1019
	0.0161	0.1010
	0.0161	0.0910
	0.0180	0.0934
		0.0922
		0.0934
	Average	0.0169
L. N. Suthers Victor Chemical Co., Chicago, Ill.	0.0168	0.0970
	0.0158	0.0994
	Average	0.0162

The recovery of fluorine in the baking powder samples, as well as in the pure fluorides, indicates clearly the possibilities of this method, or it might be said of any volatilization method. An experienced analyst should have no difficulty in obtaining an average recovery of from 90–92 per cent of the fluorine in the sample.

The use of a factor in placing the recovery of fluorine on a 100 per cent

basis is indicated. The suggestion has been made that before proceeding to the analysis of a sample of baking powder this factor be determined by the analyst on a pure sample of sodium fluoride, the factor so determined to be applied to the result of the determination.

The method described is the most accurate, the easiest of manipulation, and, in experienced hands, the most reliable of any method for the determination of fluorine that has come to the attention of the associate referee.

TABLE 2.

*Results obtained on pure samples of calcium and sodium fluoride.*

COLLABORATOR	MATERIAL	NO OF DETER- MINATIONS	FLUORINE CALCU- LATED	FLUORINE FOUND		TOTAL RECOVERY
				Maximum	Minimum	
W. E. Stokes*			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Royal Baking Powder Co., Brooklyn, N. Y.	NaF	12	45.24	43.43	38.9	90.0
Milton H. Kemp	NaF	3	45.24	41.3	40.17	90.43
James K. Morton	NaF	4	45.24	42.52	42.07	93.5
	CaF <sub>2</sub>	21	48.67	47.98	41.61	93.7

\* Results reported in 1922

The associate referee wishes to acknowledge his grateful appreciation to the R. B. Davis Baking Powder Company for furnishing the baking powder for this work, and to the different collaborators that assisted materially to bring about a standardization of this method.

#### RECOMMENDATIONS.

It is recommended—

(1) That the volatilization method for the determination of fluorine in baking powder, as presented in this report, be adopted as a tentative method.

(2) That the use of a factor to place the recovery of fluorine on a 100 per cent basis be made the object of future study.

## SECOND DAY. TUESDAY—MORNING SESSION.

### REPORT ON REAGENTS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Referee*.

At the Milwaukee meeting of the American Chemical Society, held in September of the present year, a period was set apart for consideration of reagent chemicals.

Two papers representing, respectively, the Bureaus of Standards and Chemistry were read. These papers outlined the instances of unsatisfactory chemicals that had been reported in recent years and suggested possible plans for overcoming the existing difficulties. The discussion was then opened to the meeting, and a number of members expressed their opinions on the question. Unfortunately, some of the leading chemical firms were not represented at the meeting, and therefore the discussion of the question from the manufacturers' standpoint was rather incomplete.

One chemist, who represented a prominent reagent manufacturer, said he could appreciate the buyer's point of view, because he himself had difficulty at times in getting good reagents from his own firm.

During the past year the Bureau of Chemistry received 160 shipments of chemicals, 18 of which were rejected. Of the 18, it was necessary, in several cases, to test at least three different lots before a satisfactory sample was found. The following detailed list will explain the reasons for the rejections:

*Potassium carbonate, C. P.*—Four samples from two well-known manufacturers were found, after drying, to contain only 92 per cent of potassium carbonate, which is 7 per cent below the U. S. P. requirement.

*Absolute ether.*—Three samples were rejected on account of high residue and foreign substances, including water and alcohol. The quality was, in fact, below that required for anaesthetic ether.

*Amyl Acetate.*—The three rejected samples were either contaminated from dusty bottles or were found low in ester value.

*Formaldehyde.*—The two rejected samples were far below the required pharmacopeial quality in appearance and physical condition.

*Ammonium thiocyanate.*—This sample contained iron enough to give it a pink color.

*Calcium chloride.*—This salt had an alkalinity, calculated as calcium oxide, of nearly 9 per cent.

*Carbon disulfide.*—Although claimed to have been redistilled, this sample was found to be too high in residue.

*Stannous chloride.*—This salt ran too high in water- and acid-insoluble matter.

*Lead carbonate.*—The sample submitted was too high in water-soluble matter.

*Benzene.*—A carefully purified organic liquid was packed in wet bottles, demonstrating careless handling in the shipping rooms rather than in the preparation laboratory.

As compared with the previous year, the record is less encouraging, since only 4.3 per cent of the chemicals was rejected a year ago while 11.2 per cent has been sent back this year.

The apathy that has always existed among scientific bureaus toward the quality of their reagents persists. Logically, those that make no examination of their chemical stocks are having more trouble with their reagents than those that do make such tests.

This is illustrated by a recent case. One of the bureaus of the Department of Agriculture applied to the Bureau of Chemistry for a bottle of absolute ether made in Germany. It was explained that American-made ether contained a certain impurity that did not exist in the foreign ether, and for most purposes this impurity would do no harm. The exchange was effected, and it was then learned that the American ether received was part of a lot that the Bureau of Chemistry had rejected not long before as unfit for its use. There are, however, other American-made absolute ethers that are entirely acceptable.

"Analyzed chemical reagents" are very desirable if the analysis is correctly reported on the label. These label analyses not only set the amount of impurity too low, but in some cases they state it ridiculously high, as in the case of a sulfuric acid that was claimed by its label to contain 0.02-0.04 per cent of nitrogen when, as a matter of fact, it contained a very slight trace.

#### RECOMMENDATION.

It is recommended, as formerly, that the work on reagents be continued, and that the members be urged to cooperate.

### REPORT ON EGGS AND EGG PRODUCTS.

By RAYMOND HERTWIG (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

In accordance with the recommendations of the preceding referee and of the Committee on Recommendations of Referees, the referee for this year chose to study the subject of the analysis of egg noodles. As a result of this study he hoped to be able to present to the association, with a view to their adoption as tentative, the essential methods for the analysis of a noodle.

Certain methods known to the referee for determining the egg solids content of a noodle and for detecting and identifying added coloring matter were studied collaboratively. For this purpose subsamples of a ground commercial egg noodle and of a colored macaroni were sent to the collaborators for analysis. The methods submitted for study are the following:

**MOISTURE.**

Weigh 2 grams of sample into an aluminum dish previously dried and provided with a cover. Dry uncovered in vacuo at a pressure not exceeding 100 mm. mercury and at the temperature of boiling water to constant weight (approximately 5 hours). Place the cover on the dish immediately after removing from the oven, cool in a desiccator one-half hour, and weigh.

Report results of duplicate determinations for a 5 hour and at least one additional hour period of drying. Also report the pressure in the vacuum oven during the drying.

**LIPIDS AND LIPOID PHOSPHORIC ACID.**

(Devised by the referee.)

Grind the sample to pass an 80 mesh sieve. Place 10 grams of sample and 30 cc. of 70 per cent alcohol in a 200 cc. nursing bottle and set in a water bath kept at 75°–80°C. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol. Heat for 15 minutes with frequent mixing by the same rotary motion. Add 55 cc. of 95 per cent alcohol, stopper the bottle, and shake vigorously for 2 minutes. Add 85 cc. of ether, dried over sodium, and shake well for 5 minutes. The sample should now be in a fine state of division. Centrifugalize just sufficiently to throw the solid particles out of suspension but do not pack the sample too hard. Decant the liquid into a 250 cc. beaker containing some bits of broken porcelain, and rinse off the bottle neck with ether. Repeat the extraction of the sample with three successive 25 cc. portions of *washed* ether (saturated with water), shake one or two minutes each time, centrifugalize, and decant into the beaker containing the first extract. If the sample packs too firmly after centrifugalizing, loosen it with a glass rod, and should it become too dry to pack sufficiently to permit easy decantation, add a few drops of water to the ether. Evaporate the combined ether-alcohol extract to dryness on the steam bath. (Evaporation of the last drops of water is hastened by adding a little absolute alcohol.) Dry the crude lipids thus obtained in an oven at 100°C. for 45 minutes. Dissolve the dry lipids in about 15 cc. of chloroform and filter. For this filtration the following apparatus is recommended: In the top of a bell jar connected with a filter pump place the inner tube of a Knorr fat extraction apparatus (E. and A. No. 2810, 1920 Cat.), containing an asbestos pad that has been washed with alcohol and ether and dried. A tube with a removable disk is preferable. Filter the chloroform solution through this pad, receiving the filtrate in a weighed platinum dish. Carefully remove all traces of lipids from the beaker by means of a chloroform wash bottle. The filtrate should be perfectly clear. Should the asbestos become clogged, hasten the filtration by gently rubbing the surface of the pad with a glass rod. Evaporate the chloroform off on the steam bath and dry to constant weight in the water oven (approximately 60–75 minutes). Report as lipids. Saponify by warming with 5–10 cc. of 4 per cent alcoholic potassium hydroxide, evaporate to dryness, and char well in a muffle below red heat. Extract with nitric acid and determine the phosphoric acid ( $P_2O_5$ ) by the official volumetric method<sup>1</sup> and report as lipid phosphoric acid ( $P_2O_5$ ).

**FAT.**

(Devised by the referee.)

Place 2 grams of ground sample in a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir so as to moisten all particles. Add 10 cc. of hydrochloric acid, sp. gr. 1.125; mix well; immerse the beaker in a water bath held at about 65°C.; and stir at frequent intervals for 10–15 minutes, or until the proteins and starch are completely dissolved and the solution clears. Add 10 cc. of 95 per cent alcohol and cool. Transfer the

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 3

mixture to a Röhrig or a Mojonner fat extraction tube; rinse out the beaker with 25 cc. of washed ethyl ether in three portions, and shake well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. From here proceed as directed under the official Roesse-Gottlieb method for fat in milk<sup>1</sup>, re-extracting twice more with 15 cc. of each ether.

#### LIPOIDS AND LIPOID PHOSPHORIC ACID.

(Devised by O. S. Rask and I. K. Phelps, formerly of the Bureau of Chemistry, Washington, D. C.)

Treat 5 grams of the sample in a loosely stoppered 200 cc. Erlenmeyer flask with a mixture of 10 cc. of alcohol (95 per cent), 2 cc. of concentrated ammonium hydroxide, and 3 cc. of water, keeping the contents of the flask at the boiling point for 2 minutes, preferably on the steam bath. After cooling, extract the contents of the flask with three successive 25 cc. portions of ethyl ether, mixing and tamping the material thoroughly each time with a glass rod flattened at the end and pouring the extracts off by decantation into a 250 cc. beaker. Drain out the last 25 cc. portion of ether as completely as possible, add another 15 cc. portion of the same ammoniacal alcohol solution to the flask, and disintegrate the matted material as thoroughly as possible by means of the flattened glass rod, which may be left in the flask for this purpose. Return the flask to the steam bath and repeat the entire procedure, the second set of ether extracts being poured into the beaker containing the first set. Make the second treatment with the ammoniacal alcohol mixture more gradual and somewhat longer than the first, so that the ether remaining in the flask may be evaporated off and the ammoniacal alcohol brought to the required boiling point without results disastrous to the determination.

Evaporate the combined extracts to dryness on the steam bath and extract the fat from the residue left in the beaker with successive portions (5 or 6 treatments, using about 15 cc. each time) of a mixture of equal volumes of ethyl ether and petroleum ether. Collect the extracts in a tared platinum dish (do not try to filter) and evaporate to dryness on the steam bath. Dry the residue in a water-jacketed oven at the temperature of boiling water for 30–45 minutes, cool in a desiccator, and weigh.

#### LECITHIN PHOSPHORIC ACID ( $P_2O_5$ ).

Add 3 cc. of a concentrated alcoholic solution of potassium hydroxide to the fat in the dish, as obtained in the preceding method. Evaporate to dryness, char, and determine total  $P_2O_5$  by the official volumetric method.

#### EXTRACTION AND IDENTIFICATION OF ADDED COLOR IN NOODLES AND MACARONI.

##### (1) Method devised by the referee.

To 75 grams of finely ground sample in an 8 ounce nursing bottle add 110 cc. of amyl alcohol and shake well. Add 40 cc. of hydrochloric acid (1 + 1) and shake in a machine until most of the color is extracted (15–30 minutes). Centrifugalize and pour off the amyl alcohol into a 250 cc. separatory funnel. Remove the color from the alcohol fractionally by washing successively with hydrochloric acid of decreasing concentrations as 4N, N, 0.25N, 0.125N, etc., and then with water until the washings are neutral. Add an equal volume of petroleum ether to the amyl alcohol and wash again with water, washing the ether-alcohol mixture finally with dilute sodium hydroxide. Most of the egg and wheat colors, basic coal tar dyes, and some others persist in the alcohol-ether mixture. The colors in the different washings may be identified with the aid of Bull. 448, U. S. Dept. Agr., 1917.

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1920, 227.

(2) Method devised by C. F. Jablonski<sup>1</sup>, U. S. Food and Drug Inspection Station, New York City.

(3) Method of Jablonski, as modified by M. G. Wolf<sup>2</sup>, U. S. Food and Drug Inspection Station, New York City.

All the collaborating analysts are in the Bureau of Chemistry. Those who reported their results on the analysis of the egg noodle are the following:

E. H. Berry, Chicago Station;  
R. Buchanan, Washington, D. C.;  
L. H. Chernoff, Denver Station;  
R. T. Elliott, Seattle Station;  
M. L. Hitchcock, Cincinnati Station;  
J. C. Palmer, San Francisco Station;  
L. A. Salinger, Savannah Station; and  
Max Ruderman, New York Station.

Only one collaborator, P. B. Clark, San Francisco Station, reported on the methods of color extraction.

The referee wishes to thank the collaborators for the satisfactory manner in which they completed the work assigned them.

The results reported on the noodle analyses are given in the table.

#### DISCUSSION.

##### MOISTURE METHOD.

The results for moisture reported by three collaborators, which differed so widely from those obtained by the others, are probably due to other causes than differences in the moisture content of the subsamples. The procedure of the analysts, insufficient exhaust on the vacuum oven, and inefficient desiccators are more likely explanations. Careful standardization of the procedure for this determination should prevent such occasional irregular results. Precautions not given in the method submitted, which will aid in obtaining uniform results, are the following: To use dishes with tight fitting covers, to weigh out the sample accurately with the dish covered, to dry the loosely covered sample<sup>3</sup> in vacuo at a specified pressure and temperature, to cover the sample before removing from the oven, to use efficient dehydrating agents in the desiccators, and to weigh the dish after a definite period of cooling in the desiccator. The referee has incorporated these particulars in the method as given later in this report.

##### FAT METHOD.

The results for fat, with the exception of those of two collaborators, agree very well. The collaborators who commented on the method consider it entirely satisfactory.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 12.

<sup>2</sup> L. C. Mitchell and Samuel Alfend. *The Determination of Moisture in Flour. J. Assoc. Official Agr. Chemists*, 1924, 8: 76.

This particular method for determining fat in alimentary pastes and noodles has been shown by the referee to extract more fat than direct ether extraction and also to recover from a noodle practically all the fat of the component materials<sup>1</sup>. As fat extracted by this method contains only traces of phosphoric acid the indication is that the lipins are destroyed during the acid digestion. The substances extracted are probably in most part the true fats, sterols, and fatty acids that distinguish this method from the methods used to extract ether-soluble substances in their original unchanged condition. The fat, resisting the vigorous acid digestion, may also be expected to withstand decomposition during the usual manufacture and storage of noodles, but this is not thought to be the case with substances extracted by hot alcohol<sup>2</sup>, as the lipins. The method may

TABLE 1.  
*Results from collaborators.*

COLLABORATOR	MOISTURE		FAT	LIPOIDS (HERTWIG)		LIPOIDS (RASK-PHELPS)	LIPOID P <sub>2</sub> O <sub>5</sub> (HERTWIG)	LIPOID P <sub>2</sub> O <sub>5</sub> (RASK-PHELPS)
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	
Hertwig	12.26 <sup>1</sup>		4.37	4.47			0.078	
			4.37	4.52	4.57 <sup>5</sup>	4.52 <sup>8</sup>	0.079	0.080 <sup>6</sup>
	12.36 <sup>2</sup>		4.40	4.56	4.63	4.61	0.081	0.080
Hitchcock	9.71 <sup>1</sup>	9.82 <sup>3</sup>	4.33	4.51		4.54	0.067	0.073
	9.80	9.86	4.38	4.64		4.61	0.074	0.073
Palmer	12.47 <sup>1</sup>	12.37 <sup>3</sup>	4.36	4.35		4.37	0.072	0.073
	12.50	12.41	4.38	4.37		4.42	0.073	0.078
Elliott	11.68 <sup>1</sup>		4.27	4.23		4.40	0.081	0.076
	11.69		4.34	4.28		4.45	0.084	0.078
Salinger	12.77 <sup>1</sup>	12.85 <sup>4</sup>	4.20	4.33		4.33	0.068	0.071
	12.69	12.86	4.23	4.36		4.33	0.069	0.071
Berry	8.80 <sup>1</sup>	8.40 <sup>3</sup>	4.20	4.29		4.40	0.092	0.089
	8.93	8.53	4.21	4.32		4.42	0.096	0.091
Chernoff	12.02 <sup>1</sup>	12.62 <sup>3</sup>	3.81	4.24		3.50	0.058	0.063
	12.48	12.22	3.83	4.34		3.84	0.062	0.072
Buchanan	9.78 <sup>1</sup>	9.76 <sup>3</sup>	3.73 <sup>5</sup>	4.46 <sup>7</sup>	4.06 <sup>5</sup>	4.48 <sup>7</sup>	0.072 <sup>8</sup>	0.070 <sup>8</sup>
	9.70	9.76	3.79	4.47	4.12	4.54	0.076	0.070
Ruderman	11.88	11.85 <sup>3</sup>	4.39	4.63		4.53	0.066	0.068
	11.91	11.86	4.46	4.88		4.61	0.067	0.067

<sup>1</sup> 5 hour drying period.

<sup>2</sup> 6 hour drying period.

<sup>3</sup> 5 hour drying period and then 1 hour additional drying.

<sup>4</sup> 5 hour drying period and then 3 hours additional drying.

<sup>5</sup> Sample analyzed after it had become "sour".

<sup>6</sup> By simplified method.

<sup>7</sup> Sample analyzed before it had become "sour".

<sup>8</sup> Final ether-lipoid solution filtered through cotton.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 508.

<sup>2</sup> *Ibid.*, 1923, 7: 91.



be of particular value, therefore, to calculate the egg solids content of noodles in instances where the lipins are more or less decomposed owing to improper manufacture or storage.

The method has been used by Bureau of Chemistry analysts for several years. As it is simple of operation and accurate, and yields similar results in the hands of competent analysts, the referee feels justified in recommending this method for adoption as tentative for the determination of fat in noodles.

#### LIPOID AND LIPOID-PHOSPHORIC ACID METHODS.

The individual analysts, with one exception, obtained practically the same results by the two methods for lipoids (Rask-Phelps and Hertwig). This analyst reported results by the Rask-Phelps method which were very different from the other results. Omitting these results, the averages are practically the same. The results for lipid-phosphoric acid from the respective analysts by the two methods, as well as the averages of all results by the two methods, also agree very well. Judged by the results alone from this one sample, the two methods are of about equal merit.

*Results obtained by the referee before and after simplification of Hertwig method.*

BEFORE.	
LIPOIDS	LIPOID P <sub>2</sub> O <sub>5</sub>
<i>per cent</i>	<i>per cent</i>
4.47	0.078
4.52	0.079
4.56	0.081
Average 4.52	Average 0.079
AFTER.	
4.57	0.080
4.63	0.080
Average 4.60	Average 0.080

A resumé of the collaborators' comments shows that both methods are satisfactory, with a possible preference for the Hertwig method. Suggestions from Chernoff and Hitchcock to simplify the filtering of the chloroform-lipoid solution, from Buchanan and Chernoff to use U. S. P. ether instead of dry and washed ethers, and from Buchanan to shorten the drying period of the crude lipoids led the referee to try out these and other changes in the technique of the Hertwig method. The changes that proved to be of merit are the following: To grind the sample to pass a 60

mesh instead of an 80 mesh sieve, to use 5 grams of sample and one-half the quantities of the respective reagents called for, to use U. S. P. ether instead of dry and washed ethers, to bring the beaker with the crude lipoids to apparent dryness only instead of drying 45 minutes in a water oven, to filter the chloroform-lipoid solution through a plug of cotton in a funnel instead of using the more elaborate filtering apparatus, and to saponify the lipoids by dissolving them in a little chloroform and then adding the alcoholic potassium hydroxide. These minor changes leave the principles of the method as before but simplify and shorten the procedure. As thus modified, the results for two determinations are as similar to the previous results as can be expected.

The referee considers that both methods are good. He favors the Hertwig method for the following reasons:

(1) The quantity of sample to be used may be arbitrarily increased or decreased with quantitative results if a proportionate change is made in the quantities of reagents used. To obtain large quantities of lipoid extract for further analytical study may be of value in the future.

(2) Neutral extractive agents undoubtedly affect the chemical constitution of the lipoids less than alkaline extractives. Should there arise a need to study the composition of the extract, this fact would be an important consideration. The referee has also found that ammonia may have a decomposing action on lipins<sup>1</sup>.

(3) Complete extraction of a sample is surer when the sample, in powder form, is exposed to the action of the extractive than when, as a doughy mass, it is macerated with a rod in contact with the solvent. Less care and skill are necessary on the part of the analyst.

(4) To obtain lipoids free from foreign materials, it is better to dissolve the crude lipoids in chloroform and filter out foreign material than to take up the crude lipoids in ether-petroleum ether mixture and decant off without filtering.

(5) Analyses by the referee in the past of many samples of flours, alimentary pastes, and noodles by both methods gave higher results by the Hertwig method in the majority of instances. Buchanan<sup>2</sup> analyzed 117 noodles of known composition and reported results for lipoids by the two methods; 22 per cent were markedly higher, 72 per cent approximately the same, and 6 per cent somewhat lower by the Hertwig method than by the Rask-Phelps method. The lipoid-phosphoric acid was approximately the same by both methods in most instances.

As the referee considers that the lipoid and lipoid-phosphoric acid extract of a noodle is at present the most reliable index to its egg solids content, and that the Hertwig method is the most satisfactory for this determination, he is recommending this method for adoption as a tentative method.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 91

<sup>2</sup> *Ibid.*, 1924, 7: 407.

## COLOR EXTRACTION METHODS.

P. B. Clark, the only collaborator that reported on the methods for extracting and identifying color in a noodle reported the presence of Orange I and Naphthol Yellow S in the sample examined. The Hertwig method was reported by him as decidedly more satisfactory than the other two methods. The elements in favor of this method are completeness and ease of extraction, simplicity of technique and procedure, and the shortness of time involved. The referee suggests the use of 50 grams of sample, 27 cc. of dilute hydrochloric acid (1 + 1), and about 125 cc. of amyl alcohol instead of the quantities given in the method submitted.

## WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

A method of value in noodle analysis, not mentioned in preceding collaborative study, is the determination of "water-soluble protein-nitrogen precipitable by 40 per cent alcohol", devised by the referee. The method determines principally the albumin in a noodle. Ratios between this water-soluble protein-nitrogen precipitable by 40 per cent alcohol and other component substances in a noodle indicate whether it is a whole egg or commercial yolk noodle.

The method has been used for several years by many official analysts who report it satisfactory in all respects. In the paper, to which reference has been made, Buchanan states that this method "serves to distinguish a yolk noodle from a whole egg noodle", and also that "of all methods of determining the presence of egg white this method seems the best".

An egg noodle analyzed by the referee and J. C. Palmer of the San Francisco Station gave the following results:

ANALYST	WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL
R. Hertwig	<i>per cent</i> (0.155), (0.157), 0.157
J. C. Palmer	(0.153), (0.153), 0.156

The referee believes that the method has been given sufficient trial to merit his recommendation for its adoption as a tentative method.

## CONCLUSION.

As a result of the foregoing study, the writer feels in a position to compile methods for the analysis of an egg noodle. These methods are essential to the determination of the whole egg and commercial egg yolk solids

content, are accurate and easy of operation, and yield similar results in the hands of competent analysts. In his opinion they should be published in the *Book of Methods* as tentative methods of analysis. Preparatory to such a recommendation the methods are submitted in their finished form and order as follows:

## METHODS FOR THE ANALYSIS OF EGG NOODLES.

### PREPARATION OF SAMPLE.

Take sufficient strips from the lot of noodles to be analyzed to assure a representative sample, break into small pieces with the hands, and mix. Grind 300–500 grams of the mixture in a mill until all passes through a 60 mesh sieve. Keep in a sealed container to prevent moisture changes.

### MOISTURE.

#### APPARATUS.

Aluminum dish with inverted cover fitting tightly on inside; diameter about 55 mm.; height about 15 mm.

#### DETERMINATION.

Weigh a previously dried aluminum dish and cover, add approximately 2 grams of sample, cover tightly, and reweigh. Dry the loosely covered sample<sup>1</sup> in vacuo at a pressure not exceeding 100 mm. mercury and at the temperature of boiling water, to constant weight (approximately 5 hours). Press the lid firmly in the dish before removing from the oven; cool one-half hour in a desiccator containing an efficient dehydrating agent, such as calcium carbide, fresh unslaked lime, or concentrated sulfuric acid (96 per cent  $H_2SO_4$ ); and weigh. Report the loss in weight as moisture.

### ASH.

Determine ash as directed in VII, 4<sup>2</sup>, using 3–5 grams of sample.

#### CHLORIDES IN ASH AS SODIUM CHLORIDE.

Dissolve the ash in dilute nitric acid (1 + 10), filter, wash the filter paper with hot water, and determine chlorine in the combined filtrate and washings as directed under I, 16(a) or II, 16, 17. Calculate the chlorine to its equivalent of sodium chloride.

#### ORGANIC AND AMMONIACAL NITROGEN.

Determine nitrogen as directed in I, 18, 21, or 23, using 2 grams of sample.

#### WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

Place 20 grams of sample in a 200 cc. nursing bottle, add about 100 cc. of distilled water from a 200 cc. pipet, shake the bottle violently a few times to prevent lumping of the sample, and add the remainder of the 200 cc. Shake the stoppered bottle in a machine for one-half hour. The temperature of the water should not be much over 30°C. Centrifuge the bottle to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. The

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 76.

<sup>2</sup> This reference and other similar references used in these methods may be found in *Assoc. Official Agr. Chemists, Methods*, 1920.

filtrate should be practically clear. Pipet 50 cc. of the filtrate into a 125 cc. beaker-flask or Erlenmeyer flask. Add 0.6 gram of sodium chloride and dissolve. Add a small amount of finely divided filter-paper pulp or asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Allow to stand overnight. Filter the mixture through a mat of paper pulp or asbestos in a Hirsch funnel, using light suction. Wash the flask and precipitate twice with 40 per cent alcohol. Transfer the filter mat with the precipitate to a Kjeldahl flask and determine the nitrogen by the Gunning method, I, 19. Distil the ammonia over into 10 cc. of 0.1N standard acid. Make blank determinations on the reagents and the filter paper pulp or asbestos.

Prepare the paper pulp by macerating shredded loose-fiber filter paper in hot water. Remove the water by filtering and suspend in 40 per cent alcohol for use. Prepare the asbestos by igniting and rubbing through an 8 mesh sieve. The paper pulp or asbestos aids in the filtration of the albumin. Prepare 40 per cent alcohol by mixing 50 volumes of water and 35 volumes of 95 per cent alcohol.

In the presence of a small amount of sodium chloride, the 40 per cent alcoholic solution completely precipitates albumin but does not precipitate proteoses and many other proteins. The nitrogen determined by this method is an index of the amount of water-soluble albumin in the sample.

#### FAT.

Place 2 grams of sample in a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir so as to moisten all particles. Add 10 cc. of hydrochloric acid (sp. gr. 1.125), mix well, immerse the beaker in a water bath held at about 70°–80°C., and stir at frequent intervals for 15–25 minutes, or until the proteins and starch are sufficiently hydrolyzed to form a clear solution. Add 10 cc. of 95 per cent alcohol and cool. Transfer the mixture to a Röhrig or a Mojonier fat extraction tube. Rinse out the beaker into the extraction tube with 25 cc. of washed ethyl ether, in three portions, and shake the mixture well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. Let stand until the upper liquid is practically clear. Draw off through a filter consisting of a plug of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether, into a weighed 125 cc. beaker-flask containing some porcelain chips, as much as possible of the ether-fat solution. Before weighing the beaker-flask dry it in an oven at the temperature of boiling water and then allow it to stand in the air to constant weight. Re-extract the mixture remaining in the tube twice more, each time with only 15 cc. of each ether. Shake well after adding each ether. Draw off the clear ether solutions through the filter into the same flask as before and wash the tip of the spigot, the funnel, and the end of the funnel stem with a little of a mixture of the two ethers in equal parts free from suspended water. Evaporate the ethers slowly on a steam bath, and dry the fat in a boiling water oven to constant weight (approximately 75 minutes). Remove the fat flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

The moistening of the sample with alcohol prevents lumping on addition of the acid.

#### LIPIDS AND LIPOID PHOSPHORIC ACID ( $P_2O_5$ ).

Add 15 cc. of 70 per cent alcohol to 5 grams of sample in a 200 cc. nursing bottle. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol and set in a water bath kept at 75°–80°C. Heat for 15 minutes with frequent mixing by the same rotary motion. Add 27 cc. of 95 per cent alcohol, stopper the bottle, and shake vigorously for 2 minutes. Cool, add 45 cc. of U. S. P. ether, and shake well for 5 minutes. The sample should now be in a fine state of division. Centrifugalize just sufficiently to throw the solid particles out of suspension but do not pack the sample

too firmly. Decant the liquid into a 250 cc. beaker containing some bits of broken porcelain and rinse off the bottle neck with ether. Re-extract the sample with three successive 20 cc. portions of U. S. P. ether, shake 1-2 minutes each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining moisture on the sides of the beaker by placing in a boiling water oven for 5 minutes. Dissolve the dry extract in about 15 cc. of chloroform and filter the solution into a previously dried and weighed platinum dish through a plug of cotton packed in the stem of a funnel. Free any solid extract adhering to the beaker with a glass rod and transfer all extract from the beaker bottom and sides through the filter into the first washings by means of chloroform from a wash bottle. Finally wash the funnel and stem tip. The filtrate should be perfectly clear. Evaporate the chloroform on a steam bath, dry the dish and contents in a boiling water oven to constant weight (approximately 75 minutes), and weigh. Report the extract as lipoids.

Dissolve the lipoids in a little chloroform, add 5-10 cc. of 4 per cent alcoholic potassium hydroxide, evaporate to dryness on the steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid to make the solution slightly acid, and filter. Determine phosphoric acid in the filtrate by the official volumetric method, I, 7. Report as lipid phosphoric acid ( $P_2O_5$ ).

#### EXTRACTION AND IDENTIFICATION OF ADDED COLOR.

Add about 125 cc. of amyl alcohol to 50 grams of sample in a 200 cc. nursing bottle, stopper, and shake well. Add 27 cc. of hydrochloric acid (1 + 1) and shake in a machine until most of the color is extracted (15-30 minutes). Centrifugalize and pour off the amyl alcohol into a 250 cc. separatory funnel. Wash the color out of the amyl alcohol fractionally by successive washings with hydrochloric acid of decreasing concentrations, as 4 N, N, 0.25 N, etc., and then with water until the washings are neutral. (A roughly approximate 4 N acid is made by diluting 300 cc. of concentrated hydrochloric acid to 1 liter, and the other concentrations by using 75 cc., 19 cc., etc., of concentrated acid, respectively.) Add an equal volume of petroleum ether (b. p. below 60°C.) to the amyl alcohol and wash again with water. Finally wash the ether-alcohol mixture with dilute sodium hydroxide. Most of the egg and wheat colors as well as basic coal tar dyes and some others persist in the amyl alcohol-ether mixture. See Chapter X and Bull. 448, U. S. Dept. of Agriculture, 1917, to aid in identification of the colors in the various washings.

Some colors are more or less colorless in acid solution and are apparent to the eye only after neutralization.

#### DETECTION OF WHOLE EGG OR COMMERCIAL YOLK SOLIDS IN NOODLES.

Calculate the following ratios as percentages:

$$(1) \frac{\text{Water-soluble protein-nitrogen precipitable by 40 per cent alcohol} \times 100}{\text{Organic and ammoniacal nitrogen}};$$

$$(2) \frac{\text{Water-soluble protein-nitrogen precipitable by 40 per cent alcohol} \times 100}{\text{Lipoids}}; \text{ and}$$

$$(3) \frac{\text{Lipoid phosphoric acid } (P_2O_5) \times 100}{\text{Water-soluble protein-nitrogen precipitable by 40 per cent alcohol}}.$$

Compare the values of these ratios with those obtained by the referee<sup>1</sup> and by Buchanan<sup>2</sup> in their analyses of noodles of known composition.

#### ESTIMATION OF THE PERCENTAGE OF EGG SOLIDS.

Calculate the percentage of egg solids in the noodle from the lipid-phosphoric acid content by means of the following formula and basic values. (The basic values are from a very limited number of analyses, but more extended investigations will probably not alter them materially.)

0.055% = lipid  $P_2O_5$  of flours, average value (dry basis),

1.38 % = lipid  $P_2O_5$  of whole eggs, average value (dry basis),

1.78 % = lipid  $P_2O_5$  of commercial yolk, average value (dry basis).

$A$  = percentage of lipid  $P_2O_5$  in noodle sample (dry basis) multiplied by 1.1 to account for an apparent loss of 10 per cent of the lipid  $P_2O_5$  of the ingredients during manufacture<sup>3</sup>.

$$\frac{(A - 0.055) 100}{1.38 - 0.055} \text{ or } (A - 0.055) 75.5 = E, \text{ or percentage of whole egg solids in noodle sample on dry basis, and}$$

$$\frac{E \times \text{percentage of dry matter of noodle}}{100} = \text{whole egg solids in original noodle sample.}$$

Likewise for samples made with commercial yolk:

$(A - 0.055) 58.0 = Y$  or percentage of commercial yolk solids in dry noodle sample, and

$$\frac{Y \times \text{percentage of dry matter of noodle}}{100} = \text{commercial yolk solids in the original sample.}$$

#### RECOMMENDATIONS.

It is recommended—

(1) That the methods compiled in this report be adopted as tentative methods for the analysis of egg noodles. These methods comprise the following:

Preparation of sample;

Moisture,

Ash;

Chlorides in ash as sodium chloride;

Organic and ammoniacal nitrogen;

Water-soluble protein-nitrogen precipitable by 40 per cent alcohol;

Fat;

Lipoids and lipid-phosphoric acid ( $P_2O_5$ ).

Extraction and identification of added color;

Detection of the presence of whole egg or commercial yolk solids; and

Estimation of the percentage of egg solids.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 84.

<sup>2</sup> *Ibid.*, 1924, 7: 407.

<sup>3</sup> *Ibid.*, 1923, 7: 91.

(2) That a chapter, entitled "Eggs and Egg Products", with a sub-head, "Egg Noodles", which will include the tentative methods enumerated in Recommendation 1, be added to the next edition of the *Book of Methods*.

(3) That the referee for the coming year study methods for the analysis of liquid, frozen, and dried eggs, giving special consideration to the following methods:

(a) For dried eggs:

Moisture;

Ash;

Fat, as of Recommendation 1;

Lipoids and lipoid-phosphoric acid, as of Recommendation 1. See also *J. Assoc. Official Agr. Chemists*, 1923, 7: 91;

Organic and ammoniacal nitrogen;

Water-soluble protein-nitrogen precipitable by 40 per cent alcohol, as of Recommendation 1. See also *J. Assoc. Official Agr. Chemists*, 1923, 7: 84, Zinc, *J. Assoc. Official Agr. Chemists*, 1922, 6: 9; 1923, 7: 41; 1921, 5: 194; and Preservatives.

(b) For frozen and liquid eggs:

The methods proposed by the referee for 1921, *J. Assoc. Official Agr. Chemists*, 1922, 6: 5;

Fat, as of Recommendation 1;

Lipoids and lipoid-phosphoric acid, as of Recommendation 1. See also *J. Assoc. Official Agr. Chemists*, 1923, 7: 91;

Organic and ammoniacal nitrogen;

Water-soluble protein-nitrogen precipitable by 40 per cent alcohol, as of Recommendation 1. See also *J. Assoc. Official Agr. Chemists*, 1923, 7: 84.

(4) That more data be collected for the basic values used in the formula to calculate egg solids in a noodle. These should include the analysis of a number of flour samples of the types used to manufacture noodles and of some samples of liquid, frozen, and dried whole eggs and commercial yolks.

(5) That data on the values of the ratios used to distinguish a whole egg noodle from a commercial yolk noodle, a flour, and a semolina be collected, summarized and given with the method recommended to detect the presence of whole egg or commercial yolk solids in a noodle.

No report on food preservatives was presented as no referee was appointed.

No report on coloring matters in foods was made by the referee.



## REPORT ON METALS IN FOODS.

By W. F. CLARKE (Bureau of Chemistry, Washington, D. C.), *Referee*.

## TIN.

No collaborative work has been carried out during the past year. However, the zinc-iron precipitation method has been studied further with a view to simplifying and extending its application.

Briefly outlined the method reads as follows:

In a 1 liter Pyrex Erlenmeyer flask destroy by acid digestion the organic matter in 50 grams of the food material under examination; dilute with 100 cc. of water; and neutralize with concentrated sodium hydroxide solution. Dilute with water to 350–400 cc., add  $8\frac{1}{2}$  cc. of concentrated sulfuric acid, and bring the solution to 80°C. on a steam bath. Add 20 grams of zinc powder (20 or 30 mesh) and let the action continue for 1 hour. Remove from the steam bath and let stand overnight. At the end of this period replace the reaction mixture on the steam bath and complete the precipitation of the tin at 80°C. by treating for 15–20 minutes with 2 grams of iron powder reduced by hydrogen. Connect the Erlenmeyer flask with a carbon dioxide tank and cool the reaction mixture under a stream of the gas. On a 5 or 6 mm. Witt plate in a glass funnel build up an asbestos nest and through it filter off the solution of mixed sulfates by decanting it from the metal residue. Remove the nest, skin off the outside layers as much as practicable, and place the remainder in the Erlenmeyer flask. By means of a suitable stopper lead a stream of carbon dioxide through the flask, and through a dropping funnel in the stopper add 150 cc. of concentrated hydrochloric acid. Cause the acid to fall, drop by drop, on the asbestos until the nest is thoroughly disintegrated, avoiding too violent a reaction. Wash down the sides of the flask, insuring the reduction of any oxidized iron and the dissolution of any adhering metals. After the violence of the reaction has diminished add the remainder of the acid in a full stream. Boil the acid until all the metal residue is dissolved, cool in ice water, and add rapidly through the dropping funnel 200 cc. of a previously boiled and cooled solution of dilute ammonia (1 + 1).

If the solution assumes a permanent greenish or yellowish tinge, add 40 cc. of hydrochloric acid (1 + 1), previously boiled and cooled. Cool the solution in ice water and titrate with approximately 0.02 N iodine solution, keeping the carbon dioxide flowing in through a tube inserted in the mouth of the flask. Standardize the iodine by means of a range of weighed samples of tin approximating 10 mg., 20 mg., 50 mg., and 100 mg. Prepare these standards as follows:

Dissolve the tin in 25 cc. of concentrated hydrochloric acid, add 100 cc. of water, and neutralize with concentrated sodium hydrochloride. Add 65 grams of anhydrous sodium sulfate to simulate the conditions of the method proper. Add  $8\frac{1}{2}$  cc. of concentrated sulfuric acid and bring the volume to 350 cc. or 400 cc. by means of added water. From this point carry out the regular procedure for the method. If the sample to be tested has a very high tin content a charge relatively smaller than 50 grams may be used, or the iodine solution may be standardized with a corresponding weight of tin.

Results obtained by the outlined method are shown as follows:

TIN TAKEN	IODINE REQUIRED	RATIO OF IODINE TO TIN	
mg.	cc.	cc.	mg
11.25	9.95	1	1.131
22.50	20.85	1	1.079
56.25	50.40	1	1.116
112.50	100.45	1	1.120
		Average	1.1115

The results indicate the accuracy obtainable. The acid digestion should be carried out with such care as necessary to prevent the loss of tin due to reduction by the charred material above the liquid level, and the subsequent volatilization of the metal. This is accomplished by lowering the flame in order to keep it from playing on the glass above the liquid or by substituting a suitably perforated asbestos board when the volume of liquid gets low. The use of a 1 liter Erlenmeyer flask for the acid digestion and throughout the entire procedure avoids the transferring of the material as would be necessary if a Kjeldahl flask were used. Previous work on this method had shown that great care was needed to avoid the oxidation of the iron after the acid was completely neutralized and during the filtration. This difficulty is avoided by cooling under carbon dioxide before filtering. Variation of the acid concentration previous to the titration showed that the end point is shifted according to the percentage acidity.

#### RECOMMENDATION.

It is recommended that the zinc-iron precipitation method for tin be studied in comparison with the Baker-Sellers method<sup>1</sup>, and that extensive collaborative work be carried out with a view to making one or both of these methods official.

#### REPORT ON ARSENIC.

By RAYMOND M. HANN (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The H. V. Farr method for the determination of arsenic<sup>2</sup>, which differs from the present tentative method<sup>3</sup> in passage of the total liberated gaseous reduction products through an impregnated disc of filter paper, has been studied independently by two collaborators. The results obtained confirmed the conclusions arrived at by the referee following collaborative study of the method.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 150

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 31.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 147.

Lewis B. Allyn, Director of the Westfield Testing and Research Laboratories, Westfield, Mass., has studied the method for arsenic determination from the factory control standpoint. Allyn writes as follows: "With the Farr apparatus, my results show an average of about 15 per cent lower than the Sanger modification. This may be partially due to the fact that I am more accustomed to read arsenic on narrow strips, rather than in the round stains given by the Farr apparatus. I think I can get a closer reading from the strips, which may be more easily compared with standards than seems possible with the Farr stains. In the second place, it would seem to me that the Farr modification has no advantage in simplicity over the Sanger modification. \* \* \* I have a feeling that the Farr apparatus is easily tipped over although I have had no accident with it".

C. K. Sirtt, assistant chemist of the MacAndrews and Forbes Company, Camden, N. J., has studied the method for the direct determination of arsenic in vegetable material. His comments follow: "We find that the materials we are working with produce such a heavy foam with the evolved hydrogen that use of such procedure is out of the question. Although we were unable to see any possible advantage of the Farr modification over the tentative Gutzeit method we ran several tests on samples prepared by the tentative method and also on standard arsenic solutions. The results were quite unsatisfactory. Quantities of arsenic of the order of 0.02 milligram gave practically no stain on the sensitized paper. Paper prepared as directed in the tentative method gave equally unsatisfactory results. The exact limit of sensitivity under our experimental conditions was not determined, but a test with 0.2 milligram of arsenic gave a fairly heavy stain".

During the present year no active collaborative work has been undertaken, since the collaborative study of the two previous years has proved that the tentative method is accurate to a tenth of a micro-milligram in the hands of experienced workers.

Correspondence with analysts indicates the desirability of a more accurate control of the evolution of hydrogen. Electrolytic decomposition of water as a source of hydrogen has been suggested. This method lends itself to very accurate control and is largely used in England and Germany. In point of accuracy it is no more sensitive than the tentative method. Raymond Hertwig, of the Bureau of Chemistry, advocates accurate control of the evolved gas by controlled immersion of the zinc surface in contact with the acid solution. Such procedure would give uniform evolution without the use of expensive and delicate electrical apparatus.

One of the outstanding causes of error in the estimation of arsenic is the tendency of the sensitized mercuric bromide paper to curl at its lower end during the passage of the gas from the evolution chamber and to give stains on both sides of the paper of varying length. The present

practice is to measure the length of both stains, average, and compare the average length with standards. This method has proved satisfactory. It would seem more desirable, however, if the stain could be obtained along one side of a perfectly flat sensitized paper. This condition has been considered by Hertwig, who suggests the use of two metal strips—one to be grooved to allow passage of the gas, the other to be flat, so that between them a strip of impregnated paper may be clamped.

Such a device would give a perfectly flat surface and a maximum period of contact with the evolved gas. A simple way of obtaining the same result would be to moisten the paper and by a suitable glass rod press it against the sides of the absorption tube. The gas passing over contains enough moisture to hold it in position.

A colorimetric method for the determination of small amounts of arsenic has recently been devised by D. Chouchak<sup>1</sup>. It may be described as follows:

Organic matter is destroyed by the usual wet combustion methods and the arsenic oxidized by bromine or nitric acid. The reagent, which is prepared from molybdic acid, sodium carbonate, quinine hydrochloride, and arsenic trioxide, gives a precipitate of quinine arsenomolybdate which is compared, after standing 15 minutes, with standards, or preferably by a colorimeter. Quantities of 0.00002 mg. As can be detected, but somewhat larger quantities must be used for accurate color comparison.

#### RECOMMENDATIONS.

It is recommended—

(1) That the Gutzeit method for arsenic, as modified to permit the use of hydrochloric acid as an alternative acid in the determination, be adopted as an official method.

(2) That study be made of the action of arsine on organic and inorganic mercury compounds in order to obtain a more sensitive, as well as a more stable agent for absorbing the evolved arsine.

#### REPORT ON PECTIN IN FRUITS AND FRUIT PRODUCTS<sup>2</sup>.

By H. J. WICHMANN (U. S. Food and Drug Inspection Station, Denver, Colo.), *Referee*.

The association made two recommendations for work on this subject this year, as follows:

(1) That further studies be made by the method of Carré and Haynes, for the determination of calcium pectate, and the method of Wichmann and Chernoff, for the determination of pectic acid, to determine the composition of pectin.

<sup>1</sup> *Ann. chim. anal. chim. appl.*, 1922, (2) 4: 138.

<sup>2</sup> Presented by R. E. Doolittle.

(2) That the methods for preparation of sample, alcohol precipitate, pectic acid, ash, sulfur in ash, total sulfur, and water-insoluble solids, submitted at the 1921 meeting for the determination of pectin in fruits and fruit products, be studied by the referee during the coming year.

The experience of the referee with the method of Carré and Haynes since the last report has not been satisfactory. The maximum quantity of pectin that can be used for a sample is exceedingly small, the washing of the precipitate is too troublesome, and, finally, the results obtained are often uncertain. The referee also questions whether the calcium pectate obtained is not really a mixture of substances precipitated by calcium and insoluble in acetic acid. These considerations have influenced him to abandon the method of Carré and Haynes in favor of his more convenient one for pectic acid developed with the assistance of L. H. Chernoff, of the Denver Station. It was decided, therefore, to concentrate on recommendation No. 2, for the present year.

A quantity of Hood River strawberries from Oregon was obtained; the berries were pulped by passing through a meat grinder, thoroughly mixed, transferred to pint glass jars, and sterilized in a pressure cooker at approximately 5 pounds pressure for 10 minutes. Samples of these berries were sent to all the stations of the Bureau of Chemistry, with the following directions:

#### PREPARATION OF SAMPLE.

The product has already been pulped, therefore it will be necessary only to mix well before weighing out the samples for analysis. Into a 1.3 liter (or larger) beaker weigh 300 grams of the well-mixed fruit, add 800 cc. of water, bring to a boil, and boil for 1 hour, replacing the water evaporated from time to time. Transfer to a 2000 cc. graduated flask, cool, and complete to volume. Filter through a folded filter. This filtrate is referred to hereafter as the sample solution.

#### WATER-INSOLUBLE SOLIDS.

*Method 1.*—Weigh 25 grams of the well-mixed product into a 400 cc. beaker. Add 200 cc. of warm water and digest on the water bath for 1 hour, stirring frequently. Dry in a water oven and weigh in a flat dish provided with a cover, a wad of absorbent cotton about 0.5 gram in weight, or a 15 cm. qualitative filter paper. Place the cotton in a funnel, firmly wedging a corner of it into the apex by means of a wire, but leaving the mass of the fiber loose. Moisten with water, filter the fruit solution through it, and wash with hot water until the water is colorless and no longer acid. The same directions apply if the filter paper is used. Replace the cotton wad or filter paper with the insoluble solids in the dish, dry to constant weight in the water oven, and weigh. Report whether paper or cotton was used and, if both, which was preferred.

*Method 2.*—Weigh 25 grams of the well-mixed product into a 400 cc. beaker, add 200 cc. of water, and boil vigorously for 30 minutes. Replace the water evaporated from time to time. Follow the directions for filtration given in Method 1.

#### ALCOHOL PRECIPITATE.

Evaporate 100 cc. of the sample solution to 20 cc. Add 1 or 2 lumps of cube sugar (more if water-insoluble substances show a tendency to separate) during the evaporation. Cool to room temperature. Add slowly from a separatory funnel and with con-

stant stirring 200 cc. of 95 per cent alcohol. Allow to stand 1 hour or overnight. Filter on a smooth qualitative filter paper (cotton or linen cloth such as is used for crude fiber has been suggested, and the referee requests that if the collaborators have tried it they report their experience), and wash the precipitate well with 95 per cent alcohol. Wash the precipitate from the paper back into the original beaker with a stream of hot water, and also wash the paper well. Evaporate the solution to 20 cc. and add 5 cc. of 10 per cent hydrochloric acid. If water-insoluble solids have separated, stir well and, if necessary, warm slightly to dissolve them. Add  $\frac{1}{2}$  gram of acid-treated and ignited asbestos. Again precipitate with 200 cc. of 95 per cent alcohol, as before. Allow to stand 1 hour and filter into a Gooch crucible provided with a *thin* asbestos mat. Wash well with alcohol of over 85 per cent strength and dry in a water oven. Weigh, ignite, and weigh again. The loss in weight is alcohol precipitate. If the collaborator prefers, the second filtration may be made on paper or cloth instead of on a Gooch. In such cases the precipitate must be transferred to a platinum dish for drying and ignition. Report the filtering medium used.

#### PECTIC ACID.

Add 2-4 lumps of cube sugar to 200 cc. of the sample solution and evaporate to 25 cc. Precipitate with 200 cc. of 95 per cent alcohol, allow to settle, filter, and wash with 95 per cent alcohol. Transfer the precipitate into the original beaker with hot water and wash the paper well. Evaporate the pectin solution to 40 cc. and cool. If water-insoluble substances show a tendency to form during the evaporation, stir vigorously, and, if necessary, add a few drops of 10 per cent hydrochloric acid and warm. If separation can not be prevented by these means less sample must be taken. Add from 2-5 cc. of 10 per cent sodium hydroxide (the bulk of the precipitate will indicate approximately the quantity to use after a little experience) in sufficient water to make a total volume of 50 cc. Allow to stand 15 minutes, add 40 cc. of water, 10 cc. of 10 per cent hydrochloric acid, and boil 5 minutes. Collect the pectic acid on a qualitative filter and wash with hot water. The filtration should be rapid and the filtrate clear. If it is cloudy or of a colloidal nature, the results should be discarded and the determination repeated with the addition of more alkali. Wash the pectic acid back into the beaker with a stream of hot water. Adjust to 40 cc. and repeat the saponification and precipitation just described. Then wash the pectic acid into a platinum dish, evaporate to dryness, dry to constant weight in a water oven, weigh, ignite, and weigh again. The loss in weight is pectic acid.

#### ASH.

Evaporate 100 cc. of the sample solution to dryness in a platinum dish, ash in a muffle at not above low redness, cool, and weigh.

#### ALKALINITY NUMBER OF ASH.

Determine the alkalinity with 0.1N hydrochloric acid and report as cc. N/1 acid required to neutralize 1 gram of ash. Reserve the filtrate for sulfur in ash.

#### SULFUR IN ASH.

Add 5 cc. of 10 per cent hydrochloric acid to the solution remaining after the determination of alkalinity of ash and evaporate to dryness. Heat to 110°C. for 1 hour to dehydrate any silica. Take up in 5 cc. of 10 per cent hydrochloric acid and filter, washing the paper well with hot water. Heat the filtrate to boiling and precipitate the sulfur as barium sulfate by adding 3 cc. of 10 per cent barium chloride solution. Evaporate to 100 cc. and let stand overnight. Filter on a 7 cm. paper, wash, ignite,

and weigh with the usual precautions. Use great care as the amount of precipitate is very small. Determine in duplicate. Use a Munroe crucible if possible. Report results as mg. per 100 grams, and as percentage in ash.

#### TOTAL SULFUR.

Into the largest casserole that can be placed in an available electric muffle put 1 gram of magnesium oxide and 1 gram of powdered Domino sucrose. Add 50 cc. of concentrated nitric acid and then 100 cc. of sample solution. Place the same quantity of reagents in another casserole for a blank. Evaporate on the steam bath to a pasty consistency. Place the casserole in a cold electric muffle and gradually heat to not above low redness until all nitrogen tetroxide fumes have been driven off. All the organic matter will have been destroyed. Cool, dissolve in hydrochloric acid, and filter. Adjust the acidity so that the solution contains 0.5–1 gram of free hydrochloric acid, and precipitate the sulfate as barium sulfate from the boiling solution. Evaporate to 100 cc. and allow to stand overnight. Filter, wash, ignite, and weigh as usual. If possible, filter on a Munroe crucible. This determination should be made in a room free from sulfur fumes. Especial attention should be given to the blank since the reagents used are rarely free from sulfur.

The results received from collaborators are embodied in the table. Certain comments on the results and changes in the methods suggested by these results and also by the experiences of the referee and his co-workers follow.

#### COMMENTS AND CHANGES IN METHODS.

##### PREPARATION OF SAMPLE.

The sample had been pulped previous to sending to collaborators. The procedure for the preparation of the sample recommended by the referee is as follows:

Pulp the sample by passing it through a meat grinder, being careful not to crush seeds or cause a loss of the liquid portions. Mix well. Weigh into a 1.3–2.0 liter beaker 300 grams of the well-mixed fruit, add 800 cc. of water, bring to a boil, and boil 1 hour, replacing the water evaporated from time to time. Transfer to a 2000 cc. graduated flask, cool, and complete to volume. Filter through a folded filter. This filtrate is referred to hereafter as the sample solution.

##### ASH.

With one or two exceptions the results are quite uniform and satisfactory. Of course, the ashing should be done at low temperature and in as short a time as possible to prevent loss of potassium salts by volatilization.

##### ALKALINITY NUMBER.

This value is, of course, influenced by the quantity of ash found and the conditions of ashing. The results, with one exception, are satisfactory.

## WATER-INSOLUBLE SOLIDS.

From the results of the collaborators it appears that cotton or filter paper is equally satisfactory as a filtering medium. It has been the experience of the referee that the results by the paper method will average slightly higher, perhaps due to the fact that the filter paper retains finer particles than the cotton. There may be more chance of clogging if paper is employed, but if judgment is used and the sample reduced in certain cases the analyst will have little trouble.

The referee has found, and the majority of the collaborators seem to agree with him, that Method 2 gives slightly lower results than Method 1. This, apparently, is due to the more vigorous treatment by boiling. This difference is more pronounced in the case of fruits with higher water-insoluble solids than are found in the strawberry. The tissue of the fruit should be broken up as much as possible to allow all water-soluble substances to be removed, and this seems to be better accomplished by the boiling method. The referee would recommend, therefore, that the method for water-insoluble solids be made to read as follows:

Weigh 25 grams of the well-mixed product into a 400 cc. beaker. (In the case of fruits with high insoluble solids as, for example, raspberries, a lesser quantity may be used to advantage.) Add 200 cc. of water and boil vigorously for 30 minutes. Replace the water evaporated from time to time. Dry in a water oven and weigh in a flat dish provided with a cover, a wad of absorbent cotton, or a 15 cm. qualitative filter paper. Place the cotton in a funnel by means of a wire, but leave the mass of the fiber loose. Moisten with water, filter the fruit solution through it, and wash with hot water until the wash water is colorless and no longer acid. The same directions apply if the filter paper is used. Replace the cotton wad or filter paper with the insoluble solids in the dish, dry to constant weight in the water oven, and weigh.

## ALCOHOL PRECIPITATE.

One collaborator obtained rather high results, and two collaborators low results. The reason for this is not apparent. Usually slightly higher results are obtained if the second filtration is made on paper. This may be due to more complete elimination of impurities by means of the Gooch. If the percentage of alcohol precipitate is high the Gooch crucible may become clogged and filtration almost stop. In such cases, paper for the second filtration is preferable. The volume of the precipitate in the first precipitation is often a good indicator as to choice of paper or Gooch. The final directions for alcohol precipitate recommended by the referee are as follows:

Evaporate 100 cc. of the sample solution to 20 cc. after adding 1 or 2 lumps of cube sugar, if sugar is not already present. (More sugar may have to be added if water-insoluble substances separate during the evaporation.) Cool to room temperature. Add slowly and with constant stirring 200 cc. of 95 per cent alcohol. Allow to stand 1 hour or overnight. Filter on a smooth qualitative paper and wash the precipitate well with 95 per cent alcohol. Wash the precipitate back into the original beaker with





a stream of hot water. Wash the paper well. Evaporate the solution to 20 cc. and add 5 cc. of 10 per cent hydrochloric acid. If water-insoluble solids have separated, stir well and, if necessary, warm slightly to dissolve. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, add  $\frac{1}{2}$  gram of acid-treated and ignited asbestos. Again precipitate with 200 cc. of 95 per cent alcohol as before. Allow to stand 1 hour and filter into a Gooch crucible provided with a *thin* asbestos mat. Wash well with alcohol of over 85 per cent strength, suck dry, and dry in a water oven. Weigh, ignite, and weigh again. The loss in weight is alcohol precipitate. If the quantity of the alcohol precipitate on the first precipitation appears to be large, it is better to filter the second time on paper to avoid the clogging of the Gooch. Wash the precipitate well with the wash alcohol to remove all the hydrochloric acid. Then wash the precipitate into a platinum dish for drying and ignition. Exercise care so that no loss of precipitate occurs at any point, as in many cases it may be so nearly colorless as to be almost invisible.

#### PECTIC ACID.

It will be observed that some collaborators obtained lower results than others. Work done by Chernoff may furnish an explanation of these discrepancies. Pectic acid is insoluble in water containing a small quantity of electrolyte. But it does become partly soluble in excess of pure hot water. If the washing of the pectic acid is carried too far the filter becomes clogged and there is a loss of pectic acid. On the other hand, if the pectic acid is washed insufficiently, the hydrochloric acid remaining with the pectic acid will char it on drying and cause low results. The referee tested the filtrate for acid, and as soon as there was no more acid than would correspond to 0.5 cc. of 0.1N alkali in 50 cc. of filtrate, ceased washing. A piece of litmus paper is convenient for making this test. A greater quantity than 400–500 cc. of total filtrate is not necessary. It was found that a loss of 0.01–0.04 per cent of pectic acid occurred when washing was continued till the filtrate amounted to 800 cc. Chernoff has shown that a more serious loss may occur if the saponification is conducted at too high a temperature. He found that at a temperature of 30°C. or over, increasingly larger losses occur, until at boiling temperature no pectic acid is precipitated on the addition of hydrochloric acid. It becomes necessary, therefore, to specify the conditions for the determination of pectic acid more closely. The referee recommends that the method be changed to read as follows:

Add 2–4 lumps of cube sugar to 200 cc. of sample solution, if it does not already contain sugar, and evaporate to 25 cc. Precipitate with 200 cc. of 95 per cent alcohol, allow to settle, filter, and wash with 95 per cent alcohol. Transfer the precipitate into the original beaker with hot water. Evaporate the pectin solution to 40 cc. and cool to 25°C., or below. If water-insoluble substances should separate during evaporation, stir vigorously and, if necessary, add a few drops of 10 per cent hydrochloric acid and warm. Then cool again and add from 2–5 cc. of 10 per cent sodium hydroxide (the bulk of the precipitate will indicate approximately the quantity to use) in sufficient water to make a total volume of 50 cc. Allow to stand 15 minutes, add 40 cc. of water and 10 cc. of 10 per cent hydrochloric acid, and boil 5 minutes. Filter the pectic acid

on a qualitative filter paper and wash with hot water. The filtration should be rapid and the filtrate clear. If it is cloudy or of a colloidal nature, the results should be rejected and the determination repeated with the addition of more alkali. Wash the pectic acid back into the beaker, adjust to 40 cc., cool to below 25°C., and repeat the saponification and precipitation just described. Filter and wash the pectic acid with hot water only to the point where a test of the filtrate shows a negligible quantity of acid. More than 500 cc. of total filtrate should not be necessary. Then wash the pectic acid into a platinum dish and dry on a steam bath and finally in a water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid.

#### SULFUR.

The results for sulfur determined on the ash vary quite widely, from 1-10 milligrams per 100 grams. Naturally, there would be some variations due to different conditions of ashing. Since the quantity of sulfur present is very small the factor is large, and this will tend to cause further variations. The results for total sulfur obtained are fewer, but they appear to be more uniform. In fruits where there is sufficient ash to hold the greater part of the sulfur, no great difference is found between the sulfur determined on the ash and total sulfur by the magnesium method. In the case of fruit products containing sulfured constituents, where the sulfur may be high and the ash low, the magnesium method should always be used. This method is quite tedious but more reliable. The sulfur determination in the ash may be used as a sorting out method.

#### CONCLUSION.

The methods outlined above, especially with the changes recommended, appear to be well adapted to the analysis of fruits and fruit products.

#### RECOMMENDATIONS.

It is recommended that the methods for ash, alkalinity number, water-insoluble solids, alcohol precipitate, pectic acid, sulfur in ash, and total sulfur, as finally recommended by the referee, be adopted as tentative methods by the association.

### REPORT ON THE DETERMINATION OF MOISTURE IN DRIED FRUIT<sup>1</sup>.

By R. W. HILTS<sup>2</sup> (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Referee*.

This year J. C. Palmer, of the San Francisco Station of the Bureau of Chemistry, and the referee, following the recommendation adopted last year by the association, made further attempts to adapt to dried fruits

<sup>1</sup> Presented by Raymond Hertwig

<sup>2</sup> Deceased.

the calcium carbide method for determining moisture. The apparatus was the same as that recommended by McNeil<sup>1</sup> and used last year in this work. Some hitherto unsuspected sources of error were found. It was discovered, for example, that it was almost impossible to keep the cotton which holds the powdered calcium carbide in the tube above the reaction flask thoroughly dry. Even a few milligrams of hygroscopic moisture introduce some error. To avoid this difficulty long-fibered asbestos, carefully dried, was substituted for cotton. Since ethyl alcohol possesses too high a vapor tension for accurate work, an attempt was made to use amyl alcohol as a solvent in the reaction flask. Its vapor tension is fairly low and when heated it takes up considerable water.

The amyl alcohol was first dried by refluxing for some time with finely broken calcium carbide. Even after long heating with the carbide, the amyl alcohol appeared to yield 2-3 cc. of gas when heated with powdered carbide in the apparatus for 15 minutes at 100°C. It seems possible that the amyl alcohol available contains some foreign compounds capable of reacting with carbide and yielding acetylene. When runs were tried using amyl alcohol and water alone, the yields of acetylene per gram of water, under standard conditions, would differ as much as 4 or 5 per cent. Such results are certainly not very consistent. Worst of all, it was found that when the method was tried on seeded raisins the fruit remained in hard lumps or masses; therefore the water was imperfectly abstracted by the amyl alcohol during the heating, and the results obtained were far below the truth. Even if applicable, the carbide method would entail a correction for the water formed by the neutralization of the fruit acids by calcium hydroxide. Unless some anhydrous solvent that dissolves both sugar and water and possesses a low vapor tension can be suggested, it appears that the carbide method is practically useless for fruits. At present the referee has no method depending on a principle other than drying to suggest.

It was also thought advisable to experiment further on the use of the asbestos absorbent in the drying of fruits other than raisins and currants. As stated in the 1921 report<sup>2</sup> the use of asbestos with the other fruits did not seem to be of any advantage. However, Palmer prepared two samples of dried fruit, one of seeded raisins and one of layer figs, which were submitted to a few collaborators. The samples were thoroughly ground and mixed by passing through a meat chopper three times, mixed between grindings, and then placed in glass-stoppered, paraffined bottles. Palmer also experimented on a sample of prunes. These samples were submitted to the collaborators with the following instructions:

To prevent possible evaporation, the samples should not be removed from the bottles for mixing, but suitable portions for each determination should be removed by a spatula

<sup>1</sup> U. S. Dept. Agr. Bur. Chem. Circ. 97, 1912.

<sup>2</sup> J. Assoc. Official Agr. Chemists, 1922, 6: 42

or spoon, after the upper portion of the material in the bottle has been rejected. Determine moisture in both samples in duplicate by the general method for all dried fruits adopted last year by the association<sup>1</sup>, using about 5 grams of samples and the asbestos as described for raisins in the latter part of the method description. Next repeat the determination on both samples, using about 5 grams of sample, but without asbestos, following the fore part of the general description. Report individual results. Also report the reading of the vacuum gage, if the oven is provided with the usual gage reading in inches of the mercury, and also the uncorrected barometric reading of the same date. Specify the type of vacuum oven used, i. e., whether water-jacketed or electrically heated.

The results are summarized in the accompanying table. The previous conclusion is confirmed—that the use of an absorbent, as asbestos, is necessary with fruits rich in sugar, as raisins. Otherwise the results will be appreciably low. With figs and prunes, however, the use of the absorbent does not seem actually necessary, the differences being within the limits of experimental error. This confirms the observations reported in 1921. The referee has no changes to recommend in the methods for moisture in dried fruits as adopted last year by the association.

If many determinations of moisture in dried fruits are to be made it is suggested that tin ointment boxes about 8.5 cm. in diameter, with covers, provide a cheap and satisfactory substitute for more expensive dishes.

*Results of moisture determinations in dried fruits.*

ANALYST	FIGS		SEEDED RAISINS		PRUNES		AVERAGE PRESSURE IN VACUUM OVEN
	With Asbestos	Without Asbestos	With Asbestos	Without Asbestos	With Asbestos	Without Asbestos	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>inches of mercury</i>
J. C. Palmer	24.06 23.92	23.50 23.79	17.43 17.39	16.43 16.38	24.17 24.12	23.95 23.71	2.6
H. J. Wichmann Denver, Colo.	24.08 23.91	23.93 23.89	17.47 17.34	17.02 17.00	... ..	. ..	0.5
D. H. McIntire Seattle, Wash.	24.69 24.57	24.79 24.44	17.72 17.79	17.60 17.64	... .	.... ....	0.5
Average	24.20	24.06	17.52	17.01	24.14	23.83	.

No report on canned foods was presented as no referee was appointed.

*A. J. Patten:* It has been the privilege of only one man to attend every meeting of this association. It has also been the privilege of one man to hold every position which this association has the power to confer upon its members, except one. At the first meeting of the association this man

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 117.

was elected a member of the Executive Committee. At the second meeting of the association he was elected its president. During the interim between the 5th and 6th meetings he was appointed secretary-treasurer, and at its 6th meeting he was elected to that position, an office which he held 23 years. He was then elected honorary president, a position which he has held ever since and one which we hope he will grace for many years to come. In searching through the proceedings, however, I find that he never held the position of vice-president. Whether this was a sin of commission or omission, I do not know. I trust he will tell us about that point. It gives me great pleasure to introduce Dr. H. W. Wiley.

### ADDRESS BY DR. WILEY.

MR. PRESIDENT, MEMBERS OF THE ASSOCIATION,  
LADIES AND GENTLEMEN:

I think that I can explain the omission. I can do so best, however, by telling a little story. In 1912, soon after I retired, under some stress, from the position which so many eminent men subsequently have held, I was approached by my very dear friend, Mr. Burleson—many of you may remember Mr. Burleson—who was Chief of the Post Office Department for eight years, and prior to that a very active member of the House of Representatives. He represented a State of some platitude, namely, Texas. Early in the spring, soon after my retirement from the position of Chief of the Bureau of Chemistry, Mr. Burleson came to see me, and he had a very formidable roll in his hand. He said: "I have here the signatures of 69 democratic members of the House of Representatives, asking you to become our candidate for vice-president at the Baltimore convention. We are certain to nominate Wilson, and 'Wilson and Wiley' will sweep the country". I said to him: "There are two little difficulties in the way. In the first place, I am not now a democrat, never have been one, and never expect to be one". "Well", he said, "that doesn't make any difference at all. Nobody knows what a democrat is anyhow". And he is about right. "But", I said, "there is a more serious question than that". He said: "What is that"? I said: "I have fought vice all my life and I do not care to take any office now in which vice occurs. Strike out vice and I am your man".

That is the reason I always refused to be a vice-president of this association.

I am getting a little bit embarrassed by the frequency of these occasions. I had hoped, long ago, that I might pass away at some convenient time—and I suppose if I do the office of Emeritus President will be vacant for a while—because in speaking to the same association continually it becomes necessary for me to write and commit to memory a new speech

every year. It is somewhat of a burden. If I had the advantage of speaking on a moving platform, I could make the same speech all the time. But I am like the very devout church member, to whose church it was proposed to call a new pastor. He went to church, and the text was "Simon Peter's Wife's Mother Lay Sick of a Fever". This devout church member was so struck with the ability and eloquence of the minister that when he found he was going to preach at a town not far distant the next Sunday he went over there to hear him. He again took for his text, "Simon Peter's Wife's Mother Lay Sick of a Fever". This did not, however, entirely eliminate the enthusiasm of our friend. The minister was to preach the third Sunday at a point not far distant, and so the church member went again to hear him. This time, too, he took for his text, "Simon Peter's Wife's Mother Lay Sick of a Fever". This was too much for our friend, and he rose up and said: "Great God, Pastor, isn't that old woman dead yet?"

So, I have followed the text which I have committed to memory very closely. I was much pleased with the President's paper—the part of it that I heard. As you may remember, however, he was somewhat of a borrower, for in one of my addresses a few years ago I referred to the original Colloid Chemist, and if you will remember I said that when He made the world it was without form and void. There was the Great Creator of Colloid Chemistry. Now, the colloid theory, like all other theories of chemistry, is being pushed more rapidly, perhaps, than it should be. We are always ready to adopt a new plan and magnify it beyond its merits.

I am going to break away from the beaten path of my speech, and I hope the stenographer will not fail to note it and point out some problems other than those relating particularly to agriculture.

You understand, of course, that one should pursue but one specific thing; that is the only way to master anything. I once heard a chemist qualify as an agricultural chemist, a colloid chemist, a physiological chemist, a toxicological chemist, a research chemist, and an analytical chemist. I looked at him with a great wonder, because he was the only one in existence. Well, we get narrow views. That is the great objection to an expert. He is somewhat like little Willie. His mother said: "Willie, there were two pieces of cake in the cupboard, and now there is only one. What do you say to that?" Willie said: "Well, it was rather dark, Mother, and I guess I must have overlooked that other piece". And so we who pursue specialties are apt to overlook other activities either by reason of the dark or of our imperfection of vision, and therefore we should be very observant of our scientific brethren who are working in different fields; they may happen to have much we do not possess. We are apt to become intolerant; it seems to be a trait of human nature. *Humanum est errare.*

I was much surprised when one of the members of the Executive Committee came to invite me here and asked how I was getting on with my farm. I replied, "We are suffering from oligonumismaty". He inquired what kind of an insect pest that was. He was from the south and evidently had a vision of some new kind of boll weevil. I replied, "It is worse than that; it means to the Greek 'shy of coin', to the Latin, 'res angustae', and to the Ustation, 'hard up'." He replied, "You are right, sir, you are right".

The science of chemistry is so broad that it takes in a great part of physics and mathematics. When Dr. Woodward, late president of the Carnegie Institution, one of my very dear friends and a great mathematician, discussed this problem with me, I claimed chemistry is the original science, and he claimed it is mathematics. I think we are both right, for in order to illustrate some of the great principles of chemistry we have to have the aid of the physicist and the mathematician. One of the great mathematicians and physicists, J. Willard Gibbs, of Yale, was the man that invented and demonstrated the phase rule, which was so helpful in the evolution of chemistry; he has done much research work relating to the solution of the atom. J. J. Thompson, another mathematician and physicist, was the man who discovered the electron, and in the evolution of that discovery how many eminent mathematicians and physicists have aided! Sir Ernest Rutherford, for instance, is one and now a very distinguished scientist of our own country, Dr. Robert A. Millikin, and Dr. Neils Bohr, of Denmark. They, by the rigid application of mathematics and technical skill, have demonstrated the actual mass of the electron, not only in similar atoms, like hydrogen and helium, but in very dissimilar atoms.

Thus, you see our science is not alone in the possession of all chemical wisdom. The mathematicians on the one side and the physicists on the other have given some of the most wonderful contributions to our science, and that fact should render us still more tolerant toward our brothers. So, today, I want to speak of chemistry in its service, not so much to agriculture, which is the specific function of this body, but to the public welfare in general. One of the most important things relating to the public welfare is the national defense. The late war showed chemistry in an entirely new role. When the Germans, forgetting the pact that they had signed at The Hague to refrain from the use of chlorine or other gases in warfare, on the 22nd of April at Ypres, in 1915, opened one of the cruellest and most devastating campaigns in the history of warfare, a new era was begun both as to violation of international agreements of the rules of warfare, on the one side, and as a means of offense on the other side. As a result of this attack, totally unexpected and unprepared for, 5,000 men lost their lives, and had it not been for the German timidity when one-half of the whole front of the enemy was opened to advance, and the fact



that they were afraid to go into the gassed area, as they had not yet protected themselves by gas masks, they might have won the war at once.

The gas service has now become an organized arm of warfare. We have an organized chemical warfare bloc, not only in this country, but in every country, and chemistry is occupying an advanced position in offensive and defensive warfare, ranking with the other chief branches of our service.

There are so many ways in which the chemist contributes to the public welfare that I am only touching on them. Two of the problems that were presented to the world armies were how to protect the soldiers against a warfare of this kind, and how to manufacture and use the same weapons which the enemies were using. The breach of the compact forbidding the use of gas by one army made it possible for every army to use the same weapon in its own defense. You will remember how rapidly the production of chlorine developed, necessitating the manufacture of masks to protect the soldiers, until its use as an element in gas warfare was more or less abandoned. But the Germans had something up their sleeve—and I am not saying this as a tirade against the Germans—and though their enemies were protected against chlorine they had something else—phosgene. And added to that was mustard gas and those various other gases with their branches and developments that were used during the subsequent period of the war. Therefore, we had much chemical activity to develop methods of protecting our men from these gases so as to carry on this new warfare against our enemies. In the great dye industry that the Germans had monopolized they used every one of these gases or the materials from which they were made; they had them on hand or had great factories which could manufacture them in great quantities. And so they had a great and distinct advantage over us. We scarcely knew anything of the manufacture of these gases, because we had depended all those years upon the use of German dyes. They had the monopoly not only of the dye industry but of all that went into it. The very places now occupied by the French in the Ruhr were the great centers of those factories and the sources of supply which united them all in one bloc—that wonderfully effective chemical bloc which was at the service of the German Government. So, what a disadvantage the allied armies had to overcome! They had to learn how to make these gases. Chlorine, of course, they knew how to make, but they did not make much of it. They knew how to make phosgene, but they had not much use for it. Professor Bayer, the man who first discovered mustard gas, said to his classes in 1907, "This gas will in the future prove very useful in warfare". He realized very well that a gas that blinded you after a while, but did not hurt you much at the time, was a gas that could be used very effectively a few hours before an attack by making the soldiers blind. Mustard gas was the cause of more fatalities on the allied side than all the other gases em-

ployed by Germany, but it was slow-acting and that made it all the more dangerous because people did not think to protect themselves from it as they should. Hence, mustard gas and its allied gases became the most effective engine of warfare used in the Great War. What does this mean? It means that a country like ours can no longer put aside the development of chemistry as an element of national defense.

To establish a native dye industry here should be our aim—that is, the production of dyes themselves. But why not put this country in the position of having on hand, in case of war, or in case of defense against a foreign enemy, that which it will need the most—the most powerful engine of war?

It was the discovery of gunpowder that made modern warfare possible, and it was an eventful discovery, but these noxious gases, dropped from air-ships or thrown by shell, are destined to become the most powerful armament ever discovered, far more so than gunpowder ever has been, because it is so readily distributed over so wide an area.

If, unexpectedly, we should enter another war—which God forbid—but which from the looks of things now may come very soon, if we ever have to fight for liberty, which, evidently has not been attained by the late war, these gases will be of vital importance.

Oh, how we were pleasing ourselves and congratulating ourselves by saying “No more war, at least for fifty years, until the horror of the present war is forgotten by this and the next generation”.

By going back for a moment to chaos, which was the original colloid, I might tell you the story about a doctor, an architect, and a Bolshevist, as to which of them had the oldest cult. The doctor began by saying that woman was formed by taking a rib from Adam—of course that is not true, but it is a good story to illustrate it—and therefore the science of medicine was the oldest science. “Oh, no”, said the architect, “before Adam was made there had to be a preconceived plan, and therefore the architect is older than the physician”. Whereupon the Bolshevist said, “Gentlemen, you forget who furnished the chaos”.

Now, I take it that the general development of chemistry along all these lines is the one thing for the future defense of our country, and hence we ought to pay particular attention to that and outlying elements of our profession that we do not think much about while we are pursuing these lines of development, but which are present in enormous numbers. Hence, it is becoming that even this body of chemists should have some idea of what is in store in the future.

I think I have dwelt long enough on that. We must develop all branches of chemistry in this nation, we must not depend upon brawn or money. And above all things, the development of the dye industry, it seems to me, is the most important. But there are other developments.

One is the discovery of antiseptic and prophylactic properties for the prevention, and therapeutic properties for the cure of diseases. I want to illustrate that, too. In the first place, chemists are very active in discovery. The fact that dead typhoid bacilli, when injected as serum into the blood of soldiers, will protect them absolutely against typhoid, has been proved. You will remember that in the Spanish war and also the Civil War, which I had the privilege of engaging in myself, though I did not have the privilege of dying as many of my colleagues did, typhoid was the disease which did the greatest damage in decimating our ranks. The fatalities of the late war from typhoid were so small that they couldn't be put down because of the smallness of the decimal necessary. Just think of it—typhoid destroyed!

When I was asked to go over seas, at the close of the war, I had to be immunized. I did not think I would have typhoid, but they would not take anyone, even in a civilian capacity, as I was, without being immunized. The same old story as the immunization of the world from smallpox! Because it is not universally employed, we still have smallpox and typhoid. But, oh, how comparatively little now!

In the treatment of wounds there is another discovery which chemistry gave the world—the discovery of sodium hypochlorite. We are all familiar with chloride of lime, but it is not soluble in water and hence not suitable for use in hospitals. Hypochlorite of soda is soluble in water; if you add a little salt to the solution it is stable for a long period of time, and yet when any molecule of it comes in contact with organic matter it begins to decompose, and the chlorine is set free in its nascent state. The dressing of wounds with this solution was the most effective protection of our soldiers against infection. Instead of dying of infectious diseases, such as gangrene, the hypochlorite could be injected in the track of a bullet through the body so as to disinfect the whole course of the bullet, and the recovery was most remarkable. We had hardly any gangrene, hardly any lockjaw. If a soldier was not killed, but merely wounded, the prospect was good that he would get well. And this one thing alone is of great value as a contribution to the country's defense.

I am just touching upon these phases of development in chemistry because I want to get off the beaten path.

I heard an interesting speech the other night—I did not make it, either—by Julius H. Barnes. He is now President of the Chamber of Commerce of the United States, a most exalted position at the head of all these city organizations, boards of trade, etc., all over the United States. They have just completed a most beautiful building here. Mr. Barnes addressed the Washington Board of Trade, of which I am a member—not that I am a tradesman; they take anyone who is interested in the uplift of this city. We have no vote; we are taxed without representation; we

have no voice regarding who shall tax us or what shall be done with the money we have paid; we are perfect slaves, from a political point of view. I would advise that we go on a peaceful strike, not kill anybody but just refuse to hold any office. Now, that is pretty hard, I know, for a District man to refuse to hold office. Let them go outside for District Tax Collector, if they must. Just lay down our arms. A few years ago, when the French Government asked me to be their guest to help them solve this problem in their own country, I observed all of these processes. Why, a man could not get married; he couldn't sign any document that required a notarial seal. Now, I think if we tried that here Congress might take notice. I doubt if we will do it, but we have the same provocation that the colonists had in their fight against the British Crown. I believe we are about united now, due to Lloyd George and Charles E. Hughes. I hope some day, if I keep on living indefinitely for fifty or one hundred years, to see the people here have a vote or voice in what shall be done with their money.

This Mr. Barnes made a striking speech, I think. He traced the development of this country, from the wealth point of view, and one of the things he said struck me very forcefully. He said: "There have been found in King Tutenkhamen's tomb implements of weaving and spinning which were just exactly the kind we used here until the invention of the modern machinery for this purpose". Not a single improvement had been made in the textile industry. Many of you don't remember, perhaps, but I do. I don't like to go to a tailor. I want my suits all ready-made. When one is blessed with a large degree of pulchritude he doesn't have to depend upon his clothes. And then Mr. Barnes went on describing all these great developments, closing with the peroration that no one thought that this great nation, with 110 million people and 300 billions of dollars—not marks—while England has only about one-third this amount could have started with so small a beginning.

He never said a word about the educational advantages of this country, about living longer, about public health, about the progress of science, about the development of chemistry, about the church, etc. I am afraid I am like little Willie, as far as my spiritual vision is concerned. Now, there was a great man, in a great position, feeling that our greatness depended upon wealth alone.

I don't want this country to be great in defensive warfare—or perhaps in offensive warfare alone—but I do want it to be a patriotic body of citizens, who love it not because of its wealth but because it is their country, and who will do all in their power to safeguard its future welfare. I do not want it to be proud of its wealth alone.

## SECOND DAY. TUESDAY—AFTERNOON SESSION.

### REPORT ON CEREAL FOODS<sup>1</sup>.

By C. E. MANGELS (Agricultural College, N. D.), *Referee*.

The work on cereal foods during the past year has been limited to the study of the moisture and ash methods. The methods for these determinations now listed as official are the same as those used for foods and feeding stuffs. The moisture and ash content of flour is important in the commercial handling of this commodity, and accurate and concordant results are essential. The procedure used at present is evidently not sufficiently defined to give concordant results of the accuracy required in the hands of different chemists. C. H. Bailey, of Minnesota, the preceding referee, has recommended certain amplifications which define more closely the procedure to be followed in the determination of moisture and ash.

#### MOISTURE IN CEREAL FOODS.

The vacuum method for determining moisture has been criticized recently by Snyder in a pamphlet entitled "Wheat Flour; Its Weight and Moisture Content", issued by the Millers National Federation. In the discussion of defects of the vacuum method the following statement is made: "Heating in a vacuum oven for five hours, coupled with the strong suction, may introduce three sources of error: (1) Removal of chemically combined water from the proteins, gliadin, and glutenin; (2) mechanical losses; and (3) minor losses from dissociation of other components of the flour".

#### METHODS OF DETERMINING MOISTURE.

The official method for determining moisture in foods and feeding stuffs provides for drying in vacuo or in hydrogen at the temperature of boiling water<sup>2</sup>. The vacuum method, as amplified by Bailey and given elsewhere in this report, differs from the original method in that it specifies containers, temperature as 100°C., degree of vacuum, and other procedure.

The American Association of Cereal Chemists has approved the following method of determining moisture in flour.

Place approximately 5 grams of sample in a tared metal dish, preferably an aluminum dish, with a close fitting cover, keeping cover on while weighing. Dish to be approximately one inch high by one and one-half inches in diameter. Dry to constant weight in a constant temperature oven maintained at 103°-105°C. for five to six hours with bulb of thermometer on level with sample. Replace cover, cool in a desiccator and weigh rapidly on the analytical balance. The loss in weight is calculated to per cent moisture.

This method is used by a number of commercial laboratories where vacuum equipment is not available.

<sup>1</sup> Presented by T. H. Hopper.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 71.

For saccharine and fruit products the official methods provide for drying in vacuo at 70°C., since readily decomposable substances such as levulose are present<sup>1</sup>. The official methods for these products also provide for drying at the temperature of boiling water in a water oven<sup>2</sup>.

The optional official method for determining moisture in foods and feeding stuffs provides for drying in vacuo over sulfuric acid without heat. Owing to the length of time required to obtain results, this method is not satisfactory for use in cereal laboratories where results must be obtained within a few hours.

As stated previously, the official method for foods and feeding stuffs provides for drying in a current of dry hydrogen at the temperature of boiling water. An objectionable feature of the hydrogen oven is that its proper operation requires considerable attention of the chemist. The water oven has been replaced recently by an electrically heated oven with automatic temperature control. These electric ovens are very satisfactory for routine work as they require almost no attention.

#### COLLABORATIVE WORK.

The purpose of the collaborative work was to determine (1) which method gave the most concordant results in the hands of different chemists, and (2) the difference in results between the two methods in most common use at the present time—the vacuum oven method (100°C.) and the air oven method (103°–105°C.). Drying in vacuo at 70°C. was added to determine the possibility of using a lower temperature for drying.

The collaborators were requested to determine moisture by the three methods outlined below, which in brief are (1) in vacuo at 100°C., (2) air oven 103°–105°C., and (3) in vacuo at 70°C.

#### MOISTURE METHODS.

*Method I (Proposed Method<sup>3</sup>).*—Place 2 grams of flour in a tared metal dish about 40 mm. in diameter by 25 mm. high and provided with a tight fitting cover. Dry to constant weight in a vacuum oven at a pressure of not to exceed 5 cm. of mercury and at a temperature of 100°C. Cool the dish in a desiccator and weigh as soon as the dish and contents reach the temperature of the air in the laboratory.

*Method II.*—Place approximately 5 grams of sample in a tared metal dish, preferably an aluminum dish with a tight fitting cover, keeping the cover on while weighing. The dish should be approximately 1 inch high by 1½ inches in diameter. Dry to constant weight in an oven maintained at a temperature of 103°–105°C. for 5–6 hours. Place the bulb of the thermometer on a level with the sample. Replace the cover, cool in a desiccator, and weigh rapidly on the analytical balance. The loss in weight is calculated to percentage of moisture.

*Method III.*—Place approximately 2 grams of flour in a tared metal dish about 40 mm. in diameter and 25 mm. high and provided with a tight fitting cover. Dry

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 101

<sup>2</sup> *Ibid.*, 101, 153.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 132.

TABLE 1.  
Results of moisture determinations by three different methods.

COLLABORATOR	TYPE OF VACUUM OVEN USED	SAMPLE A			SAMPLE B		
		I	II	III	I	II	III
Ruth Buchanan Bureau of Chemistry Washington, D. C.	Allen- Michael	11.69	11.17	9.98	12.99	12.19	11.12
R. C. Sherwood Minnesota Experiment Sta- tion, St. Paul, Minn.	Freas	11.88	11.39	11.45	13.05	12.76	12.30
C. L. Brooks Minnesota State Mill Minneapolis, Minn.	Freas	11.84			13.04		
W. C. Luckow American Institute of Bak- ing, Chicago, Ill.	Freas	12.03	11.34	11.51	13.16	12.72	12.47
J. R. Ulrich Tanner-Gross Co. New York, N. Y.	Freas	11.50	11.10		12.75	12.60	12.47
C. E. Mangels	Mojonnier	11.90	11.54	10.45	13.00	12.72	11.50
C. H. Briggs Howard Laboratories Minneapolis, Minn.	Special	12.07* 11.46	10.84	11.41	13.20* 12.56	11.94	11.97
W. L. Rainey Larabee Flour Mills St. Joseph, Mo.	Mojonnier	11.55†	11.13	9.80†	12.65†	12.19	10.51†
J. T. Flohill Pillsbury Flour Mills Minneapolis, Minn.	Freas	11.32	11.31	11.43	12.65	12.51	11.88
W. N. Frank Washburn Crosby Co. Minneapolis, Minn.	Freas	11.38‡	11.11	10.33	12.55‡	12.43	11.17
R. B. Potts Wichita Flour Mills Wichita, Kans.	Freas	11.84	11.34	11.51	12.76	12.35	11.75
R. C. Clark Goerz Flour Mills Newton, Kans.				10.89		12.41	
A. R. Sasse, Southwestern Milling Co. Kansas City, Mo.				10.55		11.94	
B. R. Jacobs National Cereal Products Co., Washington, D. C.				10.96		12.34	

\* Results by Method I on July 10. Lower results were obtained on July 16 when results from Methods II and III were secured. Loss may be due to opening container.

† 23 inch vacuum—Method I, 3 hours drying; Method II, 4 hours drying

‡ Covers not removed from dishes while in oven. Time of drying—Flohill and Potts report 4 hours, Briggs 4-4½ hours, others 5 hours or more, except when noted.

the uncovered sample to constant weight in a vacuum oven at a pressure of not to exceed 5 cm. of mercury and at a temperature of 70°C. Replace the cover, cool the dish in a desiccator, and weigh as soon as the dish and contents reach the temperature of the air in the laboratory.

Two samples were sent to collaborators. Sample A was flour and Sample B durum semolina. The samples were sent out in wide-mouthed cork-stoppered bottles. The lots of flour and semolina used were thoroughly mixed just previous to placing in bottles. The bottles were filled to the neck and stoppered tightly with corks that had previously been dipped in melted paraffin.

The results obtained by collaborators are given in Table 1.

#### DISCUSSION OF RESULTS.

The maximum variation in all results for the three methods is as follows: Sample A, Method I—0.75 per cent, Method II—0.99 per cent, Method III—1.71 per cent; Sample B, Method I—0.65 per cent, Method II—0.82 per cent, Method III—1.96 per cent. If, however, only results that are strictly comparative are considered, the variation is as follows: Sample A, Method I—0.71 per cent, Method II—0.44 per cent, Method III—1.53 per cent; Sample B, Method I—0.40 per cent, Method II—0.57 per cent, Method III—1.35 per cent. The results from Methods I and II show greater uniformity than those from Method III. If all results are considered, Method I shows greater uniformity than Method II, but if only strictly comparable results are considered, neither Method I nor II has any advantage.

The results from Method III are considerably lower than those from Methods I or II, and Method II is consistently somewhat lower than Method I. The average of comparable results for the three methods is as follows: Sample A, Method I—11.74 per cent, Method II—11.31 per cent, Method III—11.06 per cent; Sample B, Method I—12.94 per cent, Method II—12.55 per cent, Method III—11.77 per cent.

The results of collaborative work on moisture may be summarized as follows:

1. None of the methods shows as close concordance in results as is desirable. Methods I and II in this respect are definitely superior to Method III.

2. Drying in vacuo at 70°C., as specified in Method III, gave much lower results on the average than Methods I and II.

3. Drying in vacuo at 100°C. gave consistently higher results than drying in air at 103°–105°C.

#### WATER OVEN METHOD.

Luckow reports results obtained by drying in a water oven at the temperature of boiling water as follows: A, 9.98 per cent and B, 11.27 per



cent. It will be noted that these results are considerably lower than this analyst obtained by Method II (Table 1). The referee dried samples in a water oven at 100°C. and obtained the following results: A, 11.05 per cent and B, 12.39 per cent.

In order to determine the difference in results between the old water oven and the electric oven methods the following test was made: Three samples were dried to constant weight in an old style water jacket copper oven. The inside dimensions of this oven were approximately 9 inches by 10 inches by 8 inches. The water in the jacket was kept boiling during drying, and the temperature was taken near the samples and at the same level. The temperature was found to be only 95.5°C. The Freas electric oven was then regulated so as to give the same temperature on the top shelf (95.5°C.), and samples were dried at this temperature for comparison. The results are shown in Table 2.

TABLE 2.  
*Results of drying in electric and water ovens.*

SAMPLE	FREAS OVEN 105°C.		FREAS OVEN 95.5°C.		WATER OVEN 95.5°C.	
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
1	11.83 11.81	11.82	11.41 11.43	11.42	10.82 10.95	10.89
2	13.09 13.09	13.09	12.67 12.65	12.66	12.02 12.08	12.05
3	11.95 11.89	11.92	11.66 11.69	11.68	10.86 10.88	10.87

Drying in air in the Freas oven gave much higher results than drying in the water oven at the same temperature; the results in Table 2 also show some variation in samples dried at 105°C. and 95.5°C. in the Freas oven.

#### EFFECT OF DIFFERENT TEMPERATURES IN AIR OVEN (FREAS).

To determine the variation at different temperatures the following tests were made: The Freas oven was set at 95°, 100°, 105°, and 110°C. on successive days. Two gram portions of a sample of flour were weighed into sixteen aluminum dishes. The dishes were placed in the oven, and two dishes were withdrawn for weighing at the following intervals: 2, 3, 4, 5, 6, 7, 8, and 12 hours. The results are given in Table 3.

It will be noted that the maximum results vary directly with the temperature. There is a difference of 0.41 per cent between 95°–110°C. The differences in results between the temperatures are as follows: 95°–100°, 0.18 per cent; 100°–105°, 0.09 per cent; 105°–110°, 0.14 per cent. Pro-

TABLE 3.

*Results showing loss of moisture in drying at different temperatures\*.*

TIME IN OVEN	95°C.	100°C	105°C.	110°C
<i>hours</i>				
2	11.70†	11.86	11.85	12.05
3	11.67	11.81	11.97†	12.00
4	11.62	11.88†	11.88	12.11†
5	11.59	11.78	11.82	12.03
6	11.54	11.83	11.89	12.09
7	11.53	11.83	11.84	12.08
8	11.47	11.77	11.82	12.07
12	11.45	11.75	11.94	12.03
Maximum	11.70	11.88	11.97	12.11

\* All results are the average of two duplicates.

† Maximum result

longed heating at the lower temperature did not tend to equalize results, but there appeared to be a maximum for each temperature. Prolonged heating tended to cause slight gain in weight and a decrease in the percentage of loss. This gain in weight indicates that some oxidation is taking place. There is no evidence of decomposition due to high temperature even at 110°C., since if decomposition were taking place, a continuous loss on prolonged heating would be expected.

Lack of time prevented repetition of this test with the vacuum oven.

#### DISCUSSION OF VACUUM METHOD.

##### TYPES OF OVEN USED.

The two principal types of vacuum ovens used at present are the Freas and Mojonner. These ovens are quite different in construction. The heating unit of the Mojonner oven consists of a hot plate inside the vacuum chamber, upon which sample containers are placed. The temperature of this plate is considered the temperature of the oven. The Freas oven has no heating unit inside the vacuum chamber, and the temperature of the outside chamber is often taken as the temperature of the vacuum chamber. Some investigators claim that the temperature of the vacuum chamber is lower than that of the outside chamber, while others claim that it may be considerably higher. The other gas heated types of vacuum oven generally have a water jacket to secure even distribution of heat, and the temperature of the vacuum chamber is taken

as the temperature of the oven. The different methods of taking temperature of ovens may account for some discrepancies in results, and they should be investigated.

#### DEGREE OF VACUUM.

Buchanan reports results obtained with 0.6 cm. and 20.3 cm. pressure in the oven. The results for 0.6 cm. are used in Table 1. The following table shows the difference in results:

TABLE 4.  
*Results of drying in a vacuum oven under different pressures.*

SAMPLE	0.6 CM. PRESSURE		20.3 CM. PRESSURE		DIFFERENCE
A	<i>per cent</i> 11.70    11.69 11.67		<i>per cent</i> 11.39    11.38 11.37		<i>per cent</i> 0.31
B	13.05    12.99 12.92		12.67    12.67 12.66		0.32

The difference due to decreased vacuum is practically the same in both cases. Rainey (Table 1) reports results using a 23-inch vacuum with a Mojonnier oven. The writer, using a Mojonnier oven with less than 5 cm. pressure, secured results 0.35 per cent higher at 100°C. Table 5 shows this comparison.

TABLE 5.  
*Comparative results on drying using different pressures and temperatures.*

COLLABORATOR	SAMPLE A		SAMPLE B		TIME
	100°C.	70°C	100°C.	70°C	
C. E. Mangels Mojonnier oven under 5 cm. pressure	11.90	10.45	13.00	11.50	5 hrs. and 1 hr.
W. L. Rainey Mojonnier oven 23 inch vacuum	11.55	9.80	12.65	10.51	3 hrs.
Difference	0.35	0.65	0.35	0.99	

These comparisons indicate that the degree of vacuum is important. Vacuum ovens, it may be noted, are generally equipped at the factory with gages which register the difference between air pressure and pressure in oven, rather than actual pressure in the oven. The reading of these gages is therefore influenced by air pressure.

## MECHANICAL LOSSES.

As stated previously, Snyder has mentioned mechanical loss due to suction as a source of error in vacuum drying. A comparison was made between samples dried in alundum cones and glass tubes plugged with cotton and those dried in open dishes. The results were lower when dried in alundum or glass tubes, but since similar results were obtained with the air oven it was concluded that the difference was due to containers.

A comparison was made as follows: A disc of absorbent cotton (about 5 mm. thick) was cut so as to fit snugly in an aluminum moisture dish. The tare weight of the dish plus cotton was secured. The cotton disc was removed and a two gram sample placed in the dish. The cotton disc was then carefully replaced, so that it completely covered the flour sample. The cotton-covered samples were dried at the same time as samples without cotton, so that conditions of drying would be identical. The results of this test are given in Table 6.

TABLE 6.  
*Results of drying samples with and without cotton coverings.*

SAMPLE	ALUMINUM DISHES VACUUM 100°C	ALUMINUM DISHES (COTTON COVER—VACUUM 100°C)
A	11.90	11.95
B	13.00	13.11
C	12.19	12.21

The layer of cotton would prevent removal of flour particles by suction. The cotton-covered samples actually gave slightly higher results than the uncovered samples. These results indicate that there is no appreciable loss due to suction. A Mojonnier oven was used.

## VARIATION IN SAMPLE.

It is possible that the variation in moisture results from different collaborators might be due to change in the moisture content of the sample, although the samples sent out were very thoroughly mixed, and the glass containers should prevent loss or gain in moisture. This is noted, however, because it is a possible source of error. In commercial practice, variation in sample is probably quite important as a source of error, and the official methods should prescribe a procedure for sampling flour which would insure representative samples.

The results of moisture studies are summarized as follows:

a. Drying in a high vacuum at 100°C. gives maximum results as compared with other methods, and the results are as concordant as those obtained by other methods. Drying in vacuo at 70°C. did not give satisfactory results in collaborative work.

b. Drying in air at 103°–105°C. gave somewhat lower results than drying in vacuo at 100°C.

c. The water-jacketed copper oven is a much less efficient dryer than the Freas electric oven and gives lower results even when samples are dried to constant weight at the same temperature.

d. Temperature rather than time of drying is the important factor in drying in electric air ovens (Freas).

e. Variations in results by the vacuum method may be due to the degree of vacuum used, but errors due to mechanical loss through suction are improbable.

#### ASH.

The collaborators were requested to determine ash on the samples sent out for moisture, by the method proposed by Bailey. The method is as follows:

TABLE 7.  
*Collaborative results on the determination of ash.*

COLLABORATOR	SAMPLE A	SAMPLE B
	<i>per cent</i>	<i>per cent</i>
R. Buchanan	0.74	0.600
R. C. Sherwood	0.72	0.600
C. L. Brooks	0.72	0.600
W. C. Luckow	0.72	0.600
J. R. Ulrich	0.725	0.620
C. E. Mangels	0.731	0.595
C. H. Briggs	0.728	0.588
W. L. Rainey	0.746	0.600
J. F. Flohill	0.750	0.600
W. N. Frank	0.735	0.620
R. B. Potts	0.728	0.584
R. C. Clark	0.730	0.590
A. R. Sasse	0.738	0.607
B. R. Jacobs	0.720	0.600

#### *Proposed Official Method.*

Ignite a crucible and when cooled weigh, and rapidly weigh into it 5 grams of flour. Ignite in a muffle at approximately 550°C., taking care that no portion of the muffle becomes sufficiently hot to fuse the ash. A light gray fluffy ash should result. Cool the crucible and contents in a desiccator and weigh immediately after they reach the temperature of the air in the laboratory.

The results obtained by collaborators are given in Table 7.

The maximum variation for Sample A was 0.03 per cent and for Sample B 0.036 per cent. This method gives concordant results in the hands of different chemists. The only objection to the method is that considerable time is required for the determination.

#### RECOMMENDATIONS.

It is recommended—

(1) That the amplified vacuum method for moisture in flour and cereal products, as recommended by Bailey in 1922, be tentatively adopted as the official method of the A. O. A. C., and that the words "Replace cover" be inserted at the beginning of the second sentence. The method should read as follows:

Place 2 grams of flour in a tared metal dish about 40 mm. in diameter by 25 mm. high and provided with a tight fitting cover. Dry the uncovered sample to constant weight in a vacuum oven at a pressure of not to exceed 5 cm. of mercury and at a temperature of 100°C. Replace the cover, cool the dish in a desiccator, and weigh as soon as the dish and contents reach the temperature of the air in the laboratory.

(2) That the amplified method for ash, as recommended by Bailey in 1922, be adopted as the official method.

(3) That the factor 5.7 be made official for protein in wheat as well as in flour.

(4) That the referee study methods for moisture in flour, to determine causes of variation. The following points are suggested: (1) Types of ovens; (2) containers; (3) temperature; (4) degree of vacuum; and (5) size of sample.

(5) That the referee study the possibility of developing a rapid optional method for ash that will be suitable for routine and commercial laboratories.

(6) That the referee study methods of sampling flour for analysis and devise a uniform and practical procedure for sampling.

(7) That since the recent work of Gortner and his associates indicates that glutenin is the most important protein of the wheat kernel affecting the quality of flour, an associate or special referee be named to develop a method for determining glutenin content of flour and to revise methods for protein separations in flour.

(8) That particular attention be given to moisture and ash methods during the coming year, and that if necessary studies on other methods be delayed until moisture and ash methods are perfected.

No report on the limit of accuracy in the determination of small quantities of alcohol in beers was made by the referee.

## REPORT ON VINEGAR.

By H. A. LEPPER (Bureau of Chemistry, Washington, D. C.), *Referee*.

In keeping with the recommendations of the Referee on Vinegar for 1922<sup>1</sup>, which were concurred in by Committee C and adopted<sup>2</sup>, the methods of the association<sup>3</sup> for the determination of alcohol, reducing sugars, polarization, and color were studied. A method for the determination of sulfates was also submitted to collaborative study on a sample of generator-run cider vinegar.

## ALCOHOL.

The method for the determination of alcohol directs in a mandatory manner the use of paraffin in the distillation. This requirement is obviously to prevent foaming. As it has been the experience of the referee and of others experienced in the analysis of vinegar that foaming seldom occurs, and especially as it has been noticed that some volatile constituent usually distills over when paraffin is used, the opinion of the collaborators as to its need was requested this year. Of the seven out of ten collaborators who reported on paraffin two use it and five do not; five reported that it distills over. It was the opinion of one collaborator that the paraffin that distills over affects the results, but this point has not been determined. However, in view of the possibility of an effect on the results with some samples of paraffin and the infrequent need of it, the referee deems it advisable to change the paraffin requirement from a mandatory to an optional procedure by deleting the phrase "add a small piece of paraffin", and adding the sentence, "If the sample foams sufficiently to contaminate the distillate a small piece of paraffin, preferably free of volatile constituents, may be added".

## REDUCING SUBSTANCES.

The object of these methods is to determine the non-volatile reducing substances (sugar) and to obtain results from which volatile reducing substances and sucrose can be calculated. However, with the methods as now worded it is not possible to accomplish all that is desired. As the methods to be proposed as substitutes for the existing ones differed in minor details they were sent to collaborators for trial in the following form:

*Total reducing substances before inversion.*

To 25 cc. of the sample in a 50 cc. volumetric flask add enough concentrated sodium hydroxide solution to nearly neutralize the acid. Cool, make up to the mark with water, and determine the reducing substances on 20 cc. of the solution by VII, 25<sup>4</sup>. When the amount of reducing substances is very small use 40 cc. of the solution. Express the results as invert sugar (for malt vinegar as dextrose).

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 134.

<sup>2</sup> *Ibid.*, 1923, 6: 275.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 191.

<sup>4</sup> The numbers used in these methods refer to the publication *Assoc. Official Agr. Chemists, Methods*, 1920.

*Total reducing substances after inversion.*

Invert 25 cc. of the sample in a 50 cc. volumetric flask with 2.5 cc. of concentrated hydrochloric acid as directed in VII, 14. Neutralize with concentrated sodium hydroxide and determine reducing substances as for reducing substances before inversion.

*Non-volatile reducing substances (sugar).*

Evaporate 50 cc. of the sample on the steam or water bath to a sirupy consistency, add 10 cc. of water, and evaporate again. Repeat with 10 cc. of water. Transfer the residue to a 100 cc. volumetric flask with about 50 cc. of warm water. Cool, invert with 5 cc. of concentrated hydrochloric acid as directed in VII, 14, nearly neutralize with concentrated sodium hydroxide, cool, make up to the mark with water, and determine reducing substances in 20 cc. or 40 cc., depending on amounts present, by VII, 25. Calculate the results as invert sugar (for malt vinegar as dextrose). If the results for total reducing substances before and after inversion show the absence of sucrose the inversion is unnecessary and may be omitted.

The results obtained by the collaborators using these methods, reported in Table 1, justify their adoption.

**POLARIZATION.**

Balcom and Yanovsky<sup>1</sup> investigated the effect of lead salts in solution on the polarization of vinegar. They demonstrated that the polarization of solutions from which the lead is removed differs materially from that of the same solution in which the lead remains. They also showed the change in rotation that can be obtained on malic and lactic acids and levulose when polarized in the presence of lead salts. It was apparent from their work, as they stated, "that the use of lead salts for the clarification of cider vinegar preliminary to polarization, particularly if lead is not removed from the filtrate, may lead to entirely misleading results". On this evidence the present tentative method is open to serious objection and should be dropped.

A method providing for the removal of lead before polarization was submitted to the collaborators. It follows:

To 50 cc. of vinegar add a 20 per cent solution of neutral lead acetate, 1 cc. at a time, until precipitation is complete. Filter and remove excess of lead with potassium oxalate. Filter and polarize, preferably in a 200 mm. tube, correcting for the dilution due to the lead acetate.

As the results by this method, as reported in Table 1, were far from satisfactory, the use of a decolorizing charcoal, as suggested by Balcom and Yanovsky, was believed to be worth a trial. Three samples of decolorizing carbons, kindly furnished by the producers or sales agents, namely, "Darco", Darco Sales Corporation, N. Y.; "Norit", L. A. Salomon and Brother, N. Y.; and "Nuchar", Industrial Chemical Co., N. Y., with more of the original vinegar, were sent to collaborators with the following method:

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1921, 5: 245



Decolorize 50 cc. of the sample with decolorizing carbon. Filter through a double filter and polarize in a 200 mm. tube at 20°C. Report results on the basis of a 200 mm. tube in degrees V.

Results by one collaborator were the same with each carbon,  $-0.4^{\circ}\text{V.}$ ; by another all three were  $-0.1^{\circ}\text{V.}$ , and the referee found  $-0.3^{\circ}\text{V.}$  in every case. Each carbon gave a solution free enough from color to be easily read. The theory of the use of lead in clarification, as used in sugar determinations, is to remove, if possible, all polarizing substances other than sugar. This is not the object, however, when polarizing vinegars; in fact it is desirable to remove as little as possible of any substance that rotates polarized light. The method requiring the use and subsequent removal of lead therefore is not so desirable as the one wherein decolorizing carbon is used. The adoption of the latter method is recommended.

#### COLOR.

With very light vinegars it is advantageous to measure the color in a one-inch cell. Provision should be made in the method for the use of such cell. The method would then read as follows:

Determine the depth of color in a Lovibond tintometer by good reflected daylight, using a one-half inch or one inch cell and the brewers' scale. Express the result in terms of a one-half inch cell and so state.

#### COLOR REMOVED BY FULLERS' EARTH.

The collaborators were requested to remove the color from the sample of cider vinegar by fullers' earth and also to try a proposed method on a distilled vinegar, which they had colored with caramel. The results on the percentage of color removed from cider vinegar, reported in Table 1, vary somewhat; however, they are in agreement on the essential fact that fullers' earth does not remove as much as one-half of the color from cider vinegar. This method is primarily to detect caramel coloring, and the results on distilled vinegar show it to be worthy of adoption. The method follows:

To 50 cc. of the sample add 10 grams of fullers' earth, shake at intervals of 30 minutes, and filter through a folded filter. Place as much of the filtrate as is available in a colorimetric tube and an equal volume of the original sample in a corresponding tube, make both up to 50 cc. with water, and compare the colors. Express results in percentage of color removed. As some fullers' earth is not satisfactory for this test, try the reagent on distilled vinegar that is known to be colored with caramel.

#### SULFATES.

A method for the determination of sulfates in vinegar was submitted to the collaborators, and the results, as reported in Table 1, show the method to be desirable. The method follows:

To 10 cc. of the sample, which has been filtered absolutely clear through asbestos, add 2 cc. of approximately normal hydrochloric acid, heat to boiling, add 10 cc. of hot barium chloride solution (1 gram per 100 cc.) drop by drop, and continue the boiling

for 5 minutes, keeping the volume approximately constant by adding hot water from time to time as required. Allow to stand until the supernatant liquid is clear (overnight is convenient but should not be exceeded). Filter on an ashless filter paper or a tared Munroe crucible<sup>1</sup>. Wash free from chlorides with hot water, dry, ignite at a low red heat, cool, and weigh. Express results as milligrams of sulfur trioxide ( $\text{SO}_3$ ) in 100 cc. of vinegar.

The following analysts in various Food and Drug Inspection Stations collaborated: G. H. Arner, Philadelphia, Pa.; E. H. Berry, Chicago, Ill.; E. C. Boudreaux, New Orleans, La.; L. D. Elliott, Seattle, Wash.; W. H. Heath, Buffalo, N. Y.; L. Katz, New York, N. Y.; Wm. J. McCarthy, Cincinnati, Ohio; L. A. Salinger, Savannah, Ga.; H. R. Smith, Baltimore, Md.; and W. C. Taber, San Francisco, Calif.

The referee wishes to thank the collaborators for their helpful and kind cooperation.

TABLE 1.  
*Collaborative results on samples of vinegar.*

TOTAL REDUCING SUBSTANCES			COLOR REMOVED BY FULLERS' EARTH FROM—		SULFATES	POLARIZATION 200 MM
Before Inversion	After Inversion	Non-volatile (Sugar)	Cider Vinegar	Colored Dis- tilled Vinegar		
<i>gram per 100 cc</i>	<i>gram per 100 cc</i>	<i>gram per 100 cc</i>	<i>per cent</i>	<i>per cent</i>	<i>mg per 100 cc</i>	<i>%</i>
0.49	0.51	0.34	33.4		4.6	— 1.36
0.53	0.55	0.35	43.0	97.0	4.7	— 0.7
0.50	0.51	0.35	40.0		5.7	— 0.3
0.51	0.50	0.37			5.2	— 0.67
0.50	0.54	0.35	24.5	74.0	5.3	— 1.1
0.48	0.49	0.31	35.0	(Pract.) 100	3.7	— 0.3
0.52	0.52	0.35	36.0	(Pract.) 100		— 0.6
0.50	0.51	0.34			5.5	— 0.2
0.51	0.52	0.34			4.3	— 0.7
0.48	0.48	0.33	33.0	(Pract.) 100	4.4	— 0.5

Besides studying and revising the preceding methods as directed, it seemed desirable to make a few minor changes in the wording of several of the other methods.

#### SPECIFIC GRAVITY.

The results of this determination are mainly useful in determining net contents of a sample accurately by determining the net weight and calculating the volume. As volume measurement is usually made at 20°C. it appears desirable to determine specific gravity at 20/20 in air. This change should not be made unless it is followed consistently in all the methods for other products.

<sup>1</sup> C. E. Munroe, *J. Anal. Chem.*, 1888, 2: 241; *J. Amer. Chem. Soc.*, 1909, 31: 456. See *J. Amer. Chem. Soc.*, 1909, 31: 928, for suitable solvents for removing ignited precipitate from the crucible.

## GLYCEROL.

Several years ago another analyst and the referee had occasion to make a check determination of glycerol on a cider vinegar. The results obtained by each were duplicated five or six times but a constant difference existed. As the work was done in the same laboratory an opportunity was given to compare technique. Only one difference was noted. The analyst who obtained lower results used a 6 inch dish for the evaporation of the 90 per cent alcohol extract and exposed the whole outer surface to the temperature of the bath, while the other analyst used a 4½ inch dish and exposed only as much of the outer surface to the bath as was covered by liquid on the interior. Following this clue the referee made two determinations for glycerol on the same vinegar by the official method, varying only the manner of exposing the dish during the evaporation. When the outer surface of the evaporating dish was protected by rings on the bath so that the surface exposed was smaller in circumference than the liquid inside 0.34 gram per 100 cc. was found. When the outer surface was exposed as much as possible until the several evaporations were completed 0.22 gram per 100 cc. was obtained. It is conceivable that the concentration of the glycerol on the sides of the dish as the evaporation is going on may reach 50 per cent or over in some spots and that the under side, being directly in contact with the steam at 80–90°C., would be hot enough to vaporize some glycerine. It would seem advisable, therefore, to add a precautionary sentence to the method to guard against this possibility. Following the first sentence under "Determination" (6) add "The area of the dish exposed to the bath should not be greater in circumference than that covered by the liquid inside".

## FIXED ACIDS.

The referee for last year called attention to the desirability of changing the title of this method to "Non-volatile Acid" and suggested its calculation as acetic. As no action was taken on these changes they are again called to the association's attention.

## RECOMMENDATIONS.

It is recommended—

(1) That the official method for alcohol be amended as suggested in this report.

(2) That the methods described in the text of this report for total reducing substances before and after inversion and for non-volatile reducing substances (sugar) be adopted as official, and that the methods for total reducing substances before inversion, reducing sugars before inversion after evaporation, and reducing sugars after inversion be dropped.

(3) That the present method for polarization be dropped, and that the method specifying decolorizing carbons, described in this report, be adopted as a tentative method.

(4) That the official method for color be amended as suggested in this report.

(5) That the method described under "Color Removed by Fullers' Earth" be adopted as official.

(6) That the method given in this report for the determination of sulfates be adopted as official.

(7) That the minor changes suggested in the existing methods for specific gravity and glycerol be made, that the title of the method "Fixed Acids" be changed to "Non-volatile Acids" with calculation as acetic acid instead of malic acid, and that for the sake of uniformity the word "fixed" in the method for volatile acids be changed to "non-volatile" and the words "calculated as acetic acid" be deleted.

(8) That further study be given to the method for polarization.

No report on flavors and non-alcoholic beverages was made by the referee.

### MEAT AND MEAT PRODUCTS.

By C. ROBERT MOULTON (Institute of American Meat Packers, Chicago, Ill.), *Referee*.

The referee has no general report to make on the subject of "Meat and Meat Products", but submits the following paper:

### THE AMINO ACIDS IN THE GLOBULIN-ALBUMIN FRACTION OF BEEF FLESH.

By C. ROBERT MOULTON and E. G. SIEVEKING (Agricultural Experiment Station, Columbia, Mo.).

Thirteen different samples of the water-soluble, heat-coagulable proteins from the lean of the round cut of various beef animals formed the material used in this study. The detailed methods of analysis were given in a previous paper<sup>1</sup> and so will not be repeated here. In general the methods are those devised by Van Slyke<sup>2</sup>. The material had been preserved under alcohol for various lengths of time, amounting to fifteen years in some cases. Before analysis the samples were dried, washed with ether, dried again, and allowed to become air dry.

The results of the analyses are shown in Table 1. Each analysis represents the average of one or more duplicate determinations. The water, ash, and total nitrogen of the air-dry samples are also given, as well as the

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 86

<sup>2</sup> *J. Biol. Chem.*, 1911, 10: 15, 1915, 22: 281.

TABLE 1.  
Nitrogen distribution of globulin-albumin fraction of lean beef round.  
(Percentage of total nitrogen.)

Animal. . .	562c	594	505	591	597	592	18	121	500	501	48	Bull	Frozen Beef
Age . . . . .	Birth	11 mo.	11 mo.	17 mo.	17 mo.	21 mo.	30 mo.	32 mo.	48 mo.	47 mo.	57 mo.		
Condition . . .	Good	Fat	Fat	Thin	Me- dium	Very Thin	Thin	Fat	Thin	Very Fat	Very Fat		
Ammonia nitrogen . . .	7.62	7.08	7.48	7.78	7.83	7.28	7.28	7.72	6.33	7.34	6.94	7.29	6.45
Total humin nitrogen .	3.00	2.84	2.35	2.22	2.28	2.84	3.98	3.41	3.62	3.19	3.31	2.49	1.81
Arginine nitrogen . .	13.91	13.99	13.34	12.78	12.69	12.79	12.37	14.29	13.32	11.77	14.74	12.97	13.73
Cystine nitrogen . . .	1.06	0.92	0.88	0.82	0.78	0.85	0.94	0.91	1.00	0.81	0.87	0.92	0.83
Lysine nitrogen . . . . .	11.83	14.69	13.47	14.56	13.17	13.92	13.53	14.31	14.10	14.07	15.03	13.72	14.83
Histidine nitrogen	6.03	3.47	5.53	4.26	6.71	6.89	7.60	5.55	6.30	5.32	5.61	7.23	6.57
Mono amino acid, amino ni- trogen . . . . .	59.25	56.76	56.39	55.44	55.98	56.35	54.92	53.70	49.19	56.83	53.82	57.63	58.11
Mono amino acid, non-amino nitrogen . . . . .			1.55	1.50	1.41	0.65	0.95	3.73	2.60	0.15	2.03	0.67	0.31
Total . . . . .	102.70	99.77	100.99	99.35	100.84	101.57	101.57	103.61	96.45	99.48	102.34	102.92	102.63
Cystine from sulfur deter- mination . . . . .	3.36	2.67	2.59	2.42	2.21	2.08	2.17	2.13	3.10	2.18	2.22	2.80	2.70
Water in air dry sample	10.53	10.80	8.43	8.48	8.01	9.91	15.07	13.82	16.46	7.92	12.58	11.03	8.55
Ash . . . . .	0.63	0.36	1.07	0.64	1.10	0.82	1.04	2.15	1.09	1.13	0.92	0.64	0.62
Total nitrogen . . . .	14.01	14.51	14.66	15.13	15.00	14.56	14.15	13.65	12.86	14.98	14.10	14.50	15.21

cystine nitrogen calculated from the total sulfur determination. Results from certain of these samples were reported in the previous paper and also by Thrun and Trowbridge<sup>1</sup>.

In general, the results agree with those previously reported, the differences being no greater than those shown by the results of a single analyst obtained at different times. Good checks in this work were the exception rather than the rule, the differences between duplicate determinations being frequently as large as those seen to exist between the different samples reported.

Age, or fatness, or extreme inanition (animal 592) seems to be without effect upon the amino acid distribution of this fraction of beef flesh.

Since good duplicates were not easy to obtain, and since many of the analyses add up to considerably over 100 per cent, it was thought wise to check both the method and the manipulator by means of gelatin and casein. The results obtained are shown in Table 2, in which are shown also the results of Van Slyke<sup>2</sup> on gelatin and those of Van Slyke<sup>3</sup> and

TABLE 2.  
*Nitrogen distribution in gelatin and casein*  
(Percentage of total nitrogen)

NITROGEN	GELATIN			CASEIN				
	Van Slyke	Moulton and Sieveking		Van Slyke	Crowther and Raistrick		Moulton and Sieveking	
		1	2		1	2	1	2
Ammonia nitrogen	2.25	1.58	1.87	10.27	10.15	10.35	13.24	8.97
Total humin nitrogen	0.07	0.13	0.42	1.28	1.27	1.12	1.44	1.54
Arginine nitrogen	14.70	18.09	16.12	7.41	9.73	8.71	9.96	11.97†
Cystine nitrogen	*	*	*	0.20	1.23	1.24	0.76	0.92†
Lysine nitrogen	6.32	9.78	8.21	10.30	9.39	9.86	10.55	11.34†
Histidine nitrogen	4.48	1.54	2.78	6.21	7.17	6.47	5.28	3.29†
Mono amino acid, amino nitrogen	56.30	56.51	54.64	55.81	54.06	55.46	56.23	59.00
Mono amino acid, non-amino nitrogen	14.90	15.13	11.27	7.13	6.94	7.24	5.83	3.78
Total	99.02	102.76	95.31	98.61	99.94	100.45	103.29	100.81

\* Not determined.

† On account of an accident this is but a single result.

<sup>1</sup> *J. Biol. Chem.*, 1918, **34**: 343

<sup>2</sup> *Ibid.*, 1911-12, **10**: 15.

<sup>3</sup> *Ibid.*, 1913-14, **16**: 531.

Crowther and Raistrick<sup>1</sup> on casein. The agreement is not all that could be wished. How many of the discrepancies noted are due to differences adherent in the samples, how many to the personal equation of the analyst, and how many to the limitations of the method can not be stated. While the results reported on casein contain a majority of those data diverging farthest from the average, the agreement of the other two investigators in the case of arginine, cystine, and histidine is not good. The gelatin used was very high grade material, and the casein was the usual grade furnished by chemical supply houses.

The three types of animal protein used in this work show distinct differences in the proportions of amino acids they contain. These differences are greater than those found in the results of different analysts on the same protein. The method of analysis is, therefore, very useful, but great accuracy should perhaps not be expected.

#### RECOMMENDATION.

It is recommended that cooperative work be done on this method, and that the originator of the method, as well as others experienced in its use, be included among the collaborators.

No report on the separation of meat proteins was made by the associate referee.

### COMPOSITION OF THE FLESH OF THE SQUAB AND THE PIGEON<sup>2</sup>.

By C. ROBERT MOULTON (Institute of American Meat Packers, Chicago, Ill.) and W. S. RITCHIE (Agricultural Experiment Station, Columbia, Mo.).

#### INTRODUCTION.

The claims made by squab breeders concerning the peculiar properties of squab flesh, together with the experience of the authors with the problem of protein storage, made it appear desirable to analyze the flesh of the squab and the pigeon in order to ascertain what differences exist.

#### MATERIAL AND METHODS USED.

The material used consisted of two squabs and two grown pigeons dressed for the market and delivered to the authors in good condition. All the flesh of each pair of birds was removed from the bones after the

<sup>1</sup> *Biochem. J.*, 1916, 10: 434.

<sup>2</sup> The work reported was performed in the laboratories of the Department of Agricultural Chemistry, University of Missouri. The birds were furnished by Frank H. Hollmann, Warrenton, Mo.

skin had been carefully removed. There was not a large supply of subcutaneous fat on any of the birds, although the squabs had a somewhat fatter appearance. The squabs weighed about one pound, and the pigeons were somewhat heavier. The flesh of the two squabs was composited, ground, and analyzed according to the methods in use in the laboratories. The details have been published elsewhere<sup>1</sup>. The pigeon flesh was composited in the same manner. Between one and two days had elapsed between the killing of the birds and the analysis of the flesh.

The results are shown in the accompanying table. The globulin A nitrogen is that soluble in the usual cold water extract and coagulable by half-saturated zinc sulfate solution. The globulin B nitrogen is that fraction insoluble in the cold water used but soluble in 10 per cent ammonium sulfate.

#### DISCUSSION.

From the table it is seen that the flesh of the squab is richer in ether extract and lecithin and lower in nitrogen and total phosphorus than is the flesh of the mature pigeon. When calculated to the fat-free basis the squab has 78.76 per cent water and 3.02 per cent nitrogen, while the mature pigeon has 75.92 per cent water and 3.62 per cent nitrogen. These differences would be expected owing to the age of the squab. This age effect has been pointed out by Moulton elsewhere<sup>2</sup>.

The flesh of the squab contains less water-soluble nitrogen, as well as albumin, globulin, and all other nitrogen fractions except the peptone and

*Composition of the flesh of the squab and pigeon.*

DETERMINATIONS	COMPOSITION OF FLESH		DISTRIBUTION OF NITROGEN	
	Squab	Pigeon	Squab	Pigeon
	<i>per cent</i>	<i>per cent</i>	<i>per cent of total</i>	<i>per cent of total</i>
Water . . .	72.85	72.64		
Ether extract	7.52	4.31		
Ash . . . . .	1.17	1.29		
Phosphorus . . . . .	0.234	0.272		
Ether-soluble phosphorus	0.0202	0.0114		
Equivalent lecithin . . . . .	0.537	0.314		
Total nitrogen . . . . .	2.794*	3.466		
Water-soluble solids . . . . .	5.10	6.55		
Water-soluble ash . . . . .	1.11	1.12		
Water-soluble nitrogen . . . . .	0.563	0.795	20.15	22.94
Globulin A nitrogen . . . . .	0.114	0.214	4.08	6.17
Albumin nitrogen . . . . .	0.109	0.228	3.90	6.60
Proteose nitrogen . . . . .	0.031	0.037	1.11	1.07
Peptone and peptid nitrogen . . . . .	0.111	0.043	3.97	1.24
Amino acid and extractive N . . . . .	0.198	0.273	7.09	7.88
Globulin B nitrogen . . . . .	0.153	0.213	5.48	6.15

\* Erroneously reported as 3.794 in *J. Assoc. Official Agr. Chemists*, 1922, 6: 76

<sup>1</sup> Missouri Agr. Expt. Sta. Research Bull. 55, 1922, 5; *J. Assoc. Official Agr. Chemists*, 1922, 6: 76

<sup>2</sup> *J. Biol. Chem.*, 1923, 57: 79.



peptid nitrogen. The total nitrogen is distributed in somewhat different proportions. A larger part of the nitrogen of the pigeon is soluble. Likewise, a larger part appears as globulin and albumin nitrogen, while the proportions of proteose nitrogen are about the same in the two. The amino acid and extractive nitrogen is a somewhat greater part of the total in the pigeon than in the squab, while the peptone and peptid nitrogen is greater in the squab.

On the whole, the flesh of the squab does not show a relatively greater nutritive value than does that of the pigeon. In fact, the reverse is true except with regard to the lecithin and the total fatty matter, where the squab excels.

### DECOMPOSITION OF MEAT PRODUCTS.

By K. GEORGE FALK (Harriman Research Laboratory, Roosevelt Hospital, New York City), *Associate Referee*.

Chemical methods that are applicable to the detection of spoilage in meat must depend upon a knowledge of the chemical composition of meat, fresh and in various states of spoilage, and the meaning to be attached to the terms "spoiled meat" and "spoiling meat".

The methods in use for the determination of the various constituents in meat and meat products have been described fully in the *Book of Methods*<sup>1</sup>.

Various analyses of meat have been published at different times, the official as well as other methods being used. It would serve no useful purpose to present such analyses in the present connection. They are given in various summaries and publications to which reference may be made<sup>2</sup>.

The main interest in the present connection lies in the possibility of determining spoilage in meat by following the changes in one or more of the constituents of the meat. Such changes may be brought about either by bacterial actions or by the phenomenon commonly known as "autolysis", or by both processes. Before considering the chemical nature of these changes, the significance of the term spoilage may be dealt with briefly.

Meat (as well as other foodstuffs) may be considered to be spoiled if those partaking of it are made ill and manifest certain specific symptoms. Such cases of poisoning may be due to the addition to the meat of various

<sup>1</sup> Assoc. Official Agr. Chemists, *Methods*, 1920, 209.

<sup>2</sup> W. O. Atwater and A. P. Bryant. U. S. Dept. Agr., Office Exp. Sta. Bulletin 28, rev. ed., 1906;

R. H. A. Plimmer. *Analyses and Energy Values of Foods*, 1921, 14-56, 74;

H. C. Sherman. *Food Products*, 1917, 172.

Allen's *Commercial Organic Analysis*, 4th ed., 1914, vol. 8, article on Meat and Meat Products by

W. D. Richardson, 261-466;

J. König. *Chemie der Menschlichen Nahrungs- und Genussmittel*, IV. Auflage, 1903, vol. 1, pp. 1-98, 1451-71; 1904, vol. 2, pp. 415-571; and others.

substances such as certain metallic salts, alkaloids, etc., to the fact that the animal may have fed on certain substances toxic to man, or to some action due to micro-organisms, probably bacteria. In fact, most, if not all, cases of meat poisoning are traceable to the latter source<sup>1</sup>. The animal may have been sick at the time of slaughter, in which case the meat will be infected from this source, or the meat from a healthy animal may have become infected at any time after slaughter in the various processes of handling, storage, and preparation. The actual state of the meat, its appearance, taste, and odor, as well as its chemical composition, need not reflect in any way the toxic properties that are due essentially to the bacteria present. Chemical methods have been proposed at various times to determine spoilage due to bacterial action<sup>2</sup>. In view of the system of inspecting foodstuffs in vogue in the United States at the present time, and of the care that is taken almost universally in the preparation and cooking of meats and similar materials, it is not necessary to enter minutely into the chemical aspects of this side of the problem. Two points, however, may be mentioned. The so-called ptomaines, more or less complex amino acids, are not toxic if taken by mouth, and furthermore are present in all probability only in very badly decomposed meat. The non-poisonous character of these ptomaines under the indicated conditions has been verified repeatedly. The second point relates to the fact that different strains of bacteria when acting upon meat (as an example, though this is doubtless true of other media) may cause different changes to predominate, so that if the change in content of one constituent of the meat is followed, one organism may show considerable action and another very little<sup>3</sup>. For example, the creatine-creatinine values were found to change only slightly with *Bacillus proteus* and *Bacillus paratyphosus B* but decreased markedly with *Bacillus coli communis*, *Bacillus enteritidis*, *Bacillus subtilis*, and *Streptococcus brevis*. The purine values decreased rapidly with *Bacillus coli communis* and *Bacillus enteritidis*, while with *Bacillus proteus* and *Bacillus paratyphosus B* the purine nitrogen showed a more or less irregular (accidental) variation for the first action followed by a small decrease, and *Bacillus subtilis* and *Streptococcus brevis* gave a distinct final increase in the purine, indicating a synthesis. These results indicate possibilities in the way of distinguishing various strains of bacteria by the use of synthetic media.

Since the phenomena of "meat poisoning" are due to bacterial contamination which, especially in its early stages, may be unaccompanied by marked chemical changes, and also not be reflected in the physical state and properties of the meat, the significance of spoilage here is lim-

<sup>1</sup> I. Greenwald. *Am J Pub. Health*, 1919, 9: 595

E. O. Jordan. *Food Poisoning* University of Chicago Press, 1917;

E. Hübener. *Fleischvergiftungen und Paratyphusinfektionen*, G. Fischer, Jena, 1910

<sup>2</sup> Cf. for example, J. Tillmans and H. Mildner. *Z. Nahr. Genussm.*, 1916, 32: 65; J. Tillmans, R. Strohecker, and W. Schütze. *Z. Nahr. Genussm.*, 1921, 42: 65

<sup>3</sup> K. G. Falk, E. J. Baumann, and G. McGuire. *J. Biol. Chem.*, 1919, 37: 525-46.

ited to the effects of the ingestion of the meat. The methods of food inspection and control are sufficient in the United States to insure protection against such poisonings by contaminated meat.

The term "spoiling meat", according to common usage, applies to phenomena somewhat different from those just described. The general appearance of the meat, its odor, taste, and palatability are the characteristics by which meat is ordinarily judged. It is obvious that with such criteria, no general standard exists by which the state of the meat can be ascertained. In fact, certain kinds of meat, such as venison and game birds when badly decomposed, are eaten by preference in certain localities. In this connection the possibility of poisoning does not appear to enter directly into the question of meat spoilage.

The changes in meat in this form of spoilage make themselves evident by the formation of certain products that may affect different individuals differently. Usage and custom is the important factor here, and the standards of unspoiled meat that have been developed in the United States, perhaps arbitrarily, must be the ones to be considered. They can only be approximated, however, and the chemical tests for meat spoilage of this character must necessarily be inaccurate, at least to the extent that the standards are uncertain.

Meat will decompose or spoil on keeping. This, at least, may be universally accepted. The spoiling is very rapid at moderate temperatures, 30°–40°C., and very slow at low temperatures, 0°C. and lower. Spoilage is accompanied by, and perhaps due to, chemical changes in various constituents of the meat, but not necessarily because of the presence of bacteria and their actions. These chemical changes are included under the terms "autolysis" or "autolytic changes", and occur as a rule in the sense that complex bodies break down to form simpler bodies. The question has frequently been raised whether these changes could be ascribed to enzyme actions. Undoubtedly enzymes play a part, but it is probable that in addition changes are taking place in a number of substances that are not influenced by any enzymes. The change of complex substances to simpler substances occurs frequently, especially with bodies of organic origin, because of the unstable nature of the former. The relative rates of these changes can not be predicted. It is certain, however, that increase in temperature increases the rates of the changes.

The problem of following the chemical changes in meat that lead to so-called spoilage reduces itself, then, to a study of the formation of simple substances from the more complex bodies originally present. In applying methods of analysis, it is essential to avoid the use of those that may possibly cause decompositions or changes in the substances present; they might give misleading information with reference to the changes that may have occurred and also may indicate incorrectly the presence of toxic substances in the meat. The supposed occurrence of methylguanidine in

meat suspected of having caused poisoning is a case in point. Methylguanidine is quite toxic and has been found in spoiling meat. A recent investigation<sup>1</sup> clearly brought out the fact that methylguanidine is not present in meat except, possibly, in cases of very extensive decomposition, but that it may have been formed and observed in certain analyses because of the action of reagents (such as mercuric acetate) on creatine.

Certain criteria may be stated to be desirable in the planning of chemical tests to follow changes in meat that lead to and result in spoilage. In the first place, the change to be studied should be fairly definite chemically. That is, a definite chemical constituent should be determined or a definite chemical reaction studied. This would rule out the estimation of most of the constituents present in meat that are too complex in character (perhaps because of their colloid nature) to admit of direct and accurate chemical analysis. In the second place, the reagents used and the manipulations should be such as to cause a minimum of change in the material. This condition must be borne in mind constantly because of the more or less unstable character of a number of the substances which may be present and their sensitiveness to outside influences. Another requirement is that the change which is followed should parallel the "decomposition" of the meat, even if it is not the cause of the meat being considered spoiled and unfit for food. A desirable condition, although one not essential for the purpose in view, is that the experimental determination should be comparatively simple in character. This condition may perhaps be a necessary outcome of the first two requirements stated.

The consideration of the desirable conditions for following the chemical changes that accompany the decomposition and spoiling of meat and the study of the various constituents of meat and methods for their estimation have limited the constituents or reactions which may be of value in this study to the ammonia content and the amino nitrogen content. The work done on these constituents at the Harriman Research Laboratory in connection with the work of the Division of Food and Nutrition, Medical Department, U. S. Army<sup>2</sup>, has resulted in the adoption of the ammonia content as the test most likely to give the desired information. On the other hand, the study of the bulletin, "Changes in Fresh Beef During Cold Storage Above Freezing", by Ralph Hoagland, Charles N. McBryde and Wilmer C. Powick<sup>3</sup>, indicates a preference for the amino nitrogen estimation for the purpose in view.

The determination of ammonia in meat has been simplified by the use of the permutit method of Folin and Bell<sup>4</sup>, in which ammonia is extracted from a solution by specially prepared permutit (an aluminium silicate,

<sup>1</sup> I. Greenwald. *J. Amer. Chem. Soc.*, 1919, 41: 1109. Cf. also L. Baumann and T. Ingvaldsen. *J. Biol. Chem.*, 1918, 35: 277.

<sup>2</sup> K. G. Falk. *J. Ind. Eng. Chem.*, 1919, 11: 1062

<sup>3</sup> U. S. Dept. Agr. Bull. 433, 1917.

<sup>4</sup> *J. Biol. Chem.*, 1917, 29: 329.

zeolite), then liberated by treatment with alkali, and determined by Nesslerization. The method is as follows<sup>1</sup>:

Five grams of the meat is ground finely, treated with 60 cc. of water and 20 cc. of alumina cream in a 100 cc. volumetric flask for 1 hour at room temperature, shaken occasionally, and then made up to the mark and filtered through paper. Two grams of the permutit powder is placed in a 200 cc. volumetric flask, 10-50 cc. (depending upon the ammonia content) of the filtered meat extract added, the neck of the flask washed down with about 5 cc. of water, and the flask agitated gently for about 5 minutes. The permutit will have taken up the ammonia from the solution. About 25 cc. of ammonia free water is added in order to rinse the powder to the bottom of the flask, and the liquid is then decanted. The washing is repeated with two fresh portions of 25 cc. each of water. A little water is added, then 5 cc. of 10 per cent sodium hydroxide solution, and about 100 cc. of water. The flask is placed in a beaker containing hot water for a few minutes and 10 cc. of Nessler solution is added. The solution is mixed and allowed to stand at room temperature for 10 minutes. The flask is then filled to the mark with water, and the mixture is compared with standard solutions of ammonia. The standard solutions are made up to contain 1.0 mg. of ammonia nitrogen and less, as may be required, Nesslerized, and diluted in the same way as for the determinations in the meat extracts.

The alumina cream may be prepared conveniently, as suggested by E. M. Frankel, by diluting an aluminium acetate solution, which can be procured on the market, and boiling in a large container for several days, keeping the volume fairly constant. The salt is hydrolyzed and the acetic acid boiled off. After most of the acetic acid has been removed in this way, the mixture is filtered through paper or cloth or evaporated to small bulk and then filtered. The aluminium hydroxide is washed with water a number of times in a large precipitating jar and the wash waters decanted.

Permutit suitable for the extraction of ammonia is made by The Permutit Company, 30 East 42nd Street, New York. The permutit can be regenerated after being used, but this is hardly worth while in view of the comparatively small quantities that are used.

The Nessler solution may be prepared according to the method of Folin and Denis<sup>2</sup>, as follows:

Dissolve approximately 75 grams of potassium iodide in 50 cc. of warm water, add 100 grams of mercuric iodide and stir. Dilute to 400 cc., filter, and make up to 1 liter. To 300 cc. of this solution add 200 cc. of a 10 per cent sodium hydroxide solution and 500 cc. of water and mix. This solution can be used for the Nesslerization tests.

It may be stated that the results on the determination of ammonia in meat obtained by the permutit method agreed very satisfactorily with the results by the aeration method, provided certain precautions are used in the latter. For example, the alkali used must not be concentrated, as otherwise decomposition of the meat extractives by the alkali would occur; and further, because of the weak alkali used it is necessary to allow the aeration to proceed for a longer time.

<sup>1</sup> K. G. Falk and G. McGuire. *J. Biol. Chem.*, 1919, 37: 547.

<sup>2</sup> *J. Biol. Chem.*, 1916, 26: 479.

The estimation of ammonia by the permittit method permits of extraction of the meat at low temperatures with water and the treatment of the extracts with reagents that have a minimum tendency to cause decomposition.

A few determinations of the ammonia content of meat by the above method have been published by the writer and G. McGuire<sup>1</sup>. Beef, within 24 hours of slaughter and chilled, was found to contain between 0.03 and 0.10 milligram of ammonia nitrogen per gram of meat. Standing at low temperatures, 0°–5°C., the meat became unsuitable for use after four weeks or more and then showed an ammonia nitrogen content of over 1.0 milligram per gram in every case. In some cases the value rose to 3.0 milligrams before it was necessary to discard the meat. In these cases there was considerable growth of mold. Beef kept at room temperatures (15°–25°C.) became unsuitable for consumption very much more rapidly, due in all probability to bacterial action that could not be prevented. After 24 hours, in some cases, the meat was unfit for use, even if no indications of the formation of toxic products were present. The ammonia nitrogen content in these decompositions at higher temperatures was only 0.3–0.4 milligram per gram of meat when the food was unfit to eat. It may be mentioned that the presence of molds and bacteria on the surface of a piece of meat does not mean that the interior is necessarily contaminated.

A possible interpretation of some of the results obtained may be suggested. Bacterial growth at room temperature is comparatively rapid, and the meat becomes unfit for food even with a low ammonia content. At low temperatures, bacterial growth is slow, but autolysis proceeds so that the cleavage products, such as ammonia and doubtless compounds rich in amino nitrogen, increase greatly without the formation of those products whose odor, appearance, and general flavor make the food unsuitable for use. If the meat is kept cold first, autolysis proceeding, and then is brought to room temperature, decomposition would be much more rapid because of the simpler products formed by autolysis, which would serve as nutriment for bacteria and greatly increase their growth. This may be one reason, in addition to the physical effects of the breakdown of cell walls by freezing, for the more rapid decomposition of meat that has been in cold storage for some time.

In interpreting the results of the chemical tests for following meat spoilage, it is essential to know the previous history of the meat, such as time, temperature, and condition of storage, etc. Only with such knowledge will it be possible to make a correct appraisal of the state of decomposition. This conclusion, drawn from the more or less incomplete data at hand for the ammonia content, may serve as a tentative basis for further work with the ammonia test for meat spoilage and also for such other tests as may be suggested and used.

<sup>1</sup> *J. Biol. Chem.*, 1919, 37: 549.

## REPORT ON GELATIN.

By E. H. BERRY (U. S. Food and Drug Inspection Station, Chicago, Ill.),  
*Referee.*

According to last year's recommendation further study was made of the tentative method for the determination of zinc and the alternative method for copper and zinc.

Gelatin containing known amounts of copper and zinc was prepared at a factory located in the Chicago Station territory in the following manner: To a prepared solution of 200 pounds of gelatin known to be practically free from copper and zinc, was added a solution of copper and zinc sulfates in the requisite amounts to give 30 parts per million copper and 100 parts of zinc. After thorough mixing the solution was passed through filters and over the cooling belt in the usual manner. After drying on wire trays the gelatin was ground and mixed.

Samples of this gelatin were sent to collaborators with instructions for the determination of copper and zinc by the following methods:

## COPPER AND ZINC—TENTATIVE METHOD.

*Copper.*

Hydrolize 50 grams of gelatin with 150 cc. of dilute hydrochloric acid, 1 + 3, in a covered vessel, heating about 2 hours on the steam bath. To facilitate filtration and separation from zinc and iron later, add either about  $\frac{1}{2}$  cc. of 85 per cent phosphoric acid or 2 grams of sodium phosphate or crystallized sodium ammonium phosphate and an excess of magnesia mixture. Precipitate with hydrogen sulfide in a slightly ammoniacal solution. Allow the precipitate to settle, filter, and wash with 5 per cent ammonium chloride solution saturated with hydrogen sulfide. Dissolve off the zinc and iron sulfides, magnesium, phosphate, etc., in 75 cc. of dilute hydrochloric acid (4 per cent HCl) saturated with hydrogen sulfide. Digest filter and copper sulfide with 4 cc. of concentrated sulfuric acid and sufficient nitric acid until the residue is perfectly colorless and fuming freely. Take up with water, add 5 cc. of bromine water, and boil until the bromine is completely driven off. Remove from the heat and add a slight excess of strong ammonium hydroxide. Again boil until the excess of ammonia is expelled, as shown by a change of color of the liquid and a partial precipitation. Add a slight excess of strong acetic acid (3-4 cc. of 80 per cent acid) and boil 1 minute. Cool to room temperature and add 10 cc. of 30 per cent potassium iodide solution. Titrate at once with 0.01N sodium thiosulfate solution until the brown color has become faint and then add sufficient starch indicator to produce a marked coloration. Continue the titration cautiously until the color due to free iodine has entirely vanished. Calculate to metallic copper.

*Zinc.*

Boil the filtrate from the copper determination containing the zinc to expel hydrogen sulfide, adjust the volume to about 250 cc., add a drop of methyl orange and 5 grams of ammonium chloride, and make alkaline with ammonium hydroxide. Add hydrochloric acid, drop by drop, until the reaction is faintly acid, then 10-15 cc. of 50 per cent sodium or ammonium acetate solution and pass in hydrogen sulfide for a few minutes until precipitation is complete. Allow the precipitate to settle; filter, refilter-

ing if necessary until the filtrate is clear; and wash the precipitate twice with hydrogen sulfide water. Dissolve the precipitate in the filter with a little hydrochloric acid (1 + 3), wash the filter with water, boil the filtrate and washings to expel hydrogen sulfide, cool, and add a distinct excess of bromine water. Then add 5 grams of ammonium chloride and ammonium hydroxide until the color caused by free bromine disappears. Add hydrochloric acid (1 + 3), drop by drop, until the bromine color just reappears, then add 10–15 cc. of sodium or ammonium acetate solution (50 per cent by weight) and 0.5 cc. of ferric chloride solution (10 grams per 100 cc.) or enough to precipitate all the phosphates. Boil until all the iron is precipitated. Filter while hot and wash the precipitate with water containing a little sodium acetate. Pass hydrogen sulfide into the combined filtrate and washings until all the zinc sulfide, which should be pure white, is precipitated; filter upon a tared Gooch crucible and wash with hydrogen sulfide water, containing a little ammonium nitrate. Dry the crucible and contents in an oven, ignite at a bright red heat, cool, and weigh as zinc oxide. Calculate the weight of metallic zinc, using the factor 0.8034.

*Alternative method for copper and zinc<sup>1</sup>.*

*Chicago station method for copper and zinc.*

Digest 50–100 grams of gelatin in 5 per cent sodium hydroxide on the steam bath for 1 hour in a porcelain casserole. Neutralize with hydrochloric acid, add about 25 cc. of excess concentrated acid, and digest again for 2 hours. Neutralize the acid with ammonia, adding a slight excess. Then add a small excess of acetic acid and adjust the volume to about 500 cc. Saturate with hydrogen sulfide. After the precipitate has settled, preferably overnight, filter and wash with hydrogen sulfide water. Place the filter and precipitate back in the casserole or in a Kjehdahl flask made of zinc-free glass and add 10 cc. of concentrated sulfuric acid and about 10 cc. of concentrated nitric acid. Digest until all organic matter is destroyed, adding more nitric acid from time to time. After cooling dilute with water to about 200 cc. and precipitate the copper with hydrogen sulfide. Filter off the copper sulfide, wash with hydrogen sulfide water, and determine the copper as directed in the tentative method.

*Results of copper and zinc determinations by three methods.*

COLLABORATOR	TENTATIVE METHOD		ALTERNATIVE METHOD		CHICAGO STATION METHOD	
	Copper	Zinc	Copper	Zinc	Copper	Zinc
C. R. McKee	None	56.24	None	337.4	5.9	45.0
H. Pichette U. S. Glue Co. Milwaukee, Wis.		51.40		482.0		
Walter E. Kirby Food and Drug Inspection Sta- tion, New York, N. Y.	10.1	41.6	3.2		5.7	43.4
	10.1	43.2	4.8	65.6 62.4	6.2	41.2
A. L. Fox Food and Drug Inspection Sta- tion, Chicago, Ill.	10.7	11.0	2.1	83.6	8.9	77.6
	15.9	5.6	2.1	72.0		
E. H. Berry	7.2	73.9	14.4	114.2	5.0	104.0
		95.0			5.6	92.0

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 345.



Boil off the hydrogen sulfide from the filtrate from the copper sulfide, add an excess of bromine, and boil. Add ammonium chloride, and a small quantity of ferric chloride, and precipitate the iron with ammonia. Filter, dissolve the precipitate, and reprecipitate the iron. Combine the filtrates, make acid to acetic acid, and precipitate the zinc with hydrogen sulfide. Filter off the zinc sulfide, wash with hydrogen sulfide water, dry, ignite, and weigh as zinc oxide.

Four collaborating chemists reported. Their results are given in the table. The figures refer to parts per million.

#### COMMENTS BY COLLABORATORS.

*C. R. McKee and H. Pichette.*—Tentative Method—This method is cumbersome and time-consuming, but it gives results that can be checked. Alternative Method—In filtering through the Gooch crucible in the determination of copper difficulty was experienced in getting a clear filtrate. However, this cleared on addition of formic acid. The method and results are unsatisfactory. Chicago Station Method—The preliminary work is tedious owing to difficulty of washing the gelatinous precipitate and requires the use of the filter pump. However, this method is more satisfactory than the tentative method. In the copper determination no color was produced on addition of KI, but on the addition of starch a brown color was produced that required 0.5 cc. of 0.01N thiosulfate to discharge.

*Walter E. Kirby.*—Tentative Method—It is unnecessary to heat as long as 2 hours. As soon as floating particles of insoluble keratin appear, further heating is unnecessary and even inadvisable, since there is a tendency for the hydrochloric acid to char the gelatin and cause some trouble later in filtering off the mixed sulfides. Alternative Method—The gravimetric method (for copper) is not so accurate as the volumetric for the slightest variation in the weight of the crucibles makes a great difference. The formate method (for zinc) seems to be an excellent one, and there is no trouble in filtering off the zinc sulfide, as with the other two methods. Could not a combination of the tentative method and the formate modification be used?

In comparing the results reported in the table with those reported by last year's referee<sup>1</sup>, a wide divergence will be noted. This is true of all the methods used. It is regretted that last year's report does not state the quantities of copper and zinc which the gelatin actually contained. However, the results obtained by the various analysts are far apart and the conclusion must be drawn that none of the methods are satisfactory.

Methods on the determination of copper and zinc in gelatin have been published by Raymond Hertwig and Roger M. Mehurin<sup>2</sup>. It would seem desirable in connection with next year's work to study these methods and also any other available methods of which the referee may have knowledge, in order that satisfactory details may be available. It is also important to have a method for differentiating between edible and inedible gelatins.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 136.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1923, 15: 942.

*A. J. Patten:* We are indeed fortunate at this time in having with us the Secretary of Agriculture. I know that everyone connected with the Department of Agriculture throughout the whole country has been very much impressed with his administration of this office during the years he has been the incumbent, and it is a great honor to me to introduce to you at this time the Secretary of Agriculture, Honorable Henry C. Wallace.

## ADDRESS BY THE SECRETARY OF AGRICULTURE—THE HONORABLE HENRY C. WALLACE.

MR. CHAIRMAN AND MEMBERS OF THE ASSOCIATION OF OFFICIAL  
AGRICULTURAL CHEMISTS:

It is always with a good deal of diffidence that I come before a technical body of this sort; not that you don't receive me heartily—even to the extent of pinning a badge on me which I have not earned or deserved—but in the very nature of things I am not able to contribute much to your information on the subjects in which you are so interested.

But I am always glad to come, perhaps on the theory of our good old Methodist friends when they cheerfully bear testimony so often. It tends to strengthen my own faith and perhaps to reassure you of my continued interest in the work. The longer I am in agricultural work the more I am impressed with the value of the research man, in whatever field he may be. Usually when I talk to groups of farmers I find myself spending more time on that phase of the work than on any other; I ask them to look back for 10, 15, or 20 years and note the change in agriculture that has been brought about by scientific men in the field of research. It is interesting to look back, as I often do, to the time when I was on the farm and in college and note the remarkable changes, practically all of which have come by painstaking scientific research.

So, when I come before you, I come with a feeling very strong in me of the contribution you have made in your particular field and in the practical every-day affairs of life, and of the great benefit that has come to the public as a result of your work. I am also deeply appreciative of the advantages that have come to the Department through your cooperation, directly or indirectly, through the enforcement of the regulatory acts with which it is charged.

You may be interested in some things that have happened in the Department. As you know, beginning in 1921, under the appropriation act passed that winter, we were authorized to appoint two directors, one of scientific work and one of regulatory work. The first position was filled by the appointment of Dr. E. D. Ball, and during the time he has been acting in that capacity I think he has done some things of interest to scientific men everywhere and has greatly stimulated our scientific re-

search work. He has contributed a great deal to the improvement everywhere, not only by being in more direct contact than any secretary can with the work of investigation, but by making it possible for our younger scientific men to get the stimulus and encouragement that come from contact with others—more direct contact than they have had. The establishment of our graduate schools has had very direct results; now every college is giving an opportunity to the younger men coming in—the material from which the scientific man is made—to perfect themselves while continuing the work in which they are at present engaged. And much of this work is due to the tireless efforts of Dr. Ball in speeding up the graduate school and establishing these relationships.

The second director appointed is Mr. W. G. Campbell, who for a time was Acting Chief of the Bureau of Chemistry. I am sure I could not say any words of appreciation of Mr. Campbell that would not be re-echoed by every one here. He is just getting broken into that particular work, and I am sure he is going to do great things of lasting benefit to the Department. And there is Mr. C. W. Warburton, who is in charge of extension work, in which you are interested, because it is through our extension work that the work of the scientific men is applied to the people. And then I feel very happy over our success in inducing Dr. C. A. Browne to come as the Chief of the Bureau of Chemistry. I think we have a pretty good team—a four-horse team—pulling along the lines in which you are interested.

And I come, as I have been doing, simply to express again the appreciation of the help we have received from you and also the earnest desire on the part of all of our people to fit in with your work and to help you in every way possible, to the end that we may all accomplish the most in helping the great American public.

## REPORT ON SPICES AND OTHER CONDIMENTS.

By ARTHUR E. PAUL<sup>1</sup> (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Referee*.

The work during the present year includes methods for

1. The examination of salad dressings, and
2. The determination of crude fiber in prepared mustard.

Because of the dissimilarity of the two lines of investigation, they are treated separately throughout the report.

### SALAD DRESSING.

The work on spices and other condiments last year was confined to methods for the analysis of salad dressings. The work on this subject

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<sup>1</sup> Presented by E. H. Berry.

was performed on one sample prepared in this laboratory. The results were rather discordant and not entirely satisfactory, owing to the fact, it is believed, that the dressing submitted underwent considerable separation and deterioration between the time of preparation and time of analysis.

In order to arrive at a satisfactory conclusion as to whether the methods submitted were faulty, it seemed desirable to repeat the investigation during the present year. A sample was prepared containing a smaller percentage of oil and also containing an emulsifying agent in addition to the yolk of eggs. It is the understanding of the referee that these samples reached their destination in a fairly satisfactory condition.

#### SAMPLES.

The one sample submitted to collaborators was prepared according to the following formula:

	<i>grams</i>	<i>per cent</i>
Oil	1000	47.73
Gum arabic	274	13.08
Vinegar	135	6.44
Egg yolks (7 yolks)	130	6.21
Water	509	24.30
Boric acid	5	0.23
Mustard flour	12	0.57
Sugar	10	0.48
Salt	20	0.96
Total	2095	100.00

#### METHODS.

Collaborators were requested to examine the sample by substantially the same methods of analysis as in the previous year.

#### COLLABORATORS' REPORTS.

The four collaborators that reported on this sample were the following: H. A. Lepper, Bureau of Chemistry, Washington, D. C.; E. H. Berry, Chicago, Ill.; J. H. Bornmann, Chicago, Ill.; and C. A. Greenleaf, Cincinnati, Ohio. It will be noted that the agreement with the actual composition is quite satisfactory.

#### COLLABORATORS' COMMENTS.

*H. A. Lepper.*—No difficulties were experienced with any method.

*J. H. Bornmann.*—No difficulty was experienced with any of the methods.

#### RECOMMENDATION.

It is recommended that the following methods for the examination of salad dressings be adopted as tentative methods:

TABLE 1.  
*Results on salad dressing.*

DETERMINATION	LEPPER	BERRY	BORNMANN	GREENLEAF
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total solids.....	66.12 66.21	66.13 66.22	66.24 66.17	66.58 66.38
Reducing sugar before inversion.. (as invert)	0.19 0.19	Trace	0.29 0.29	0.17 0.15
Reducing sugar after inversion . (as invert)	0.61 0.59	0.40 0.44	0.69 0.69	0.64 0.62
Sucrose.....	0.40 0.38	0.42 ...	0.38 0.38	0.45 0.45
Total acid (as acetic) . . . . .	0.7 0.8	0.70	0.72 0.70	0.70 0.70
Oil . . . . .	50.40 50.23	48.46 ..	51.72 51.49	51.92 51.33
Halphen test for cottonseed oil . . . .	.	Negative	Negative	Negative
Bellier test for peanut oil.....	.	Negative	Negative	Negative
Test for turmeric. . . . .	Negative	.	Negative	Negative
Lecithin $P_2O_4$ . . . . .	0.0568 0.0577	0.047 0.046	0.0569 0.0545	0.047
Egg yolk . . . . .	6.90 7.01	5.71 5.60	6.91 6.63	5.77 .

*Methods for the analysis of salad dressings.*

**PREPARATION OF SAMPLE.**

Before removing any portion of the sample for analysis and every time such a portion is removed, if the sample has stood for any appreciable length of time, mix until it is thoroughly homogeneous. For the various determinations take approximately the quantity directed, in each case accurately weighed. A Bailey weighing buret may be used<sup>1</sup>.

**TOTAL SOLIDS.**

Weigh 10 grams of the sample into a tared lead dish (bottle cap) having a diameter of about  $2\frac{1}{2}$  inches and containing 10-15 grams of clean dry quartz sand. Evaporate to apparent dryness on the steam bath and then dry to constant weight in a vacuum oven at the temperature of boiling water. Cool in a desiccator and weigh. Weighings on samples high in solids should be made at 1 hour intervals. (Reserve the dry material for the determination of lecithin-phosphoric acid  $P_2O_4$ .)

**REDUCING SUGARS BEFORE AND AFTER INVERSION.**

Extract the oil from 20 grams of the sample in a wide-mouthed 4 ounce bottle by adding about 80 cc. of petroleum ether, shaking, and centrifugalizing. Draw off as much as possible of the petroleum ether solution, using suction and a short-stemmed

<sup>1</sup> C. A., 1916, 10: 3003.

pipet, and repeat the treatment with petroleum ether until all the oil has been removed. This is indicated by the absence of color in the solvent. Four extractions are usually required.

Remove the petroleum ether from the residue with a current of air and transfer the residue with water to a 100 cc. graduated flask. Add 5-10 cc. of a fresh solution of metaphosphoric acid (prepared by dissolving 5 grams of the transparent lumps or sticks in cold water and making up to 100 cc.), mix thoroughly, make up to volume, and filter. Transfer 80 cc. of the filtrate, or as large an aliquot as possible, to a 100 cc. flask and, after neutralizing with a strong solution of sodium hydroxide, using phenolphthalein as indicator, cooling, and making up to the mark with water, determine the reducing sugar before inversion on an aliquot by the Murson and Walker method<sup>1</sup>.

Invert another aliquot portion and determine the reducing sugar after inversion by the same method. Calculate as invert sugar in both cases.

NOTE.—Some dressings, particularly those containing starch, can not be clarified in this manner. It is then necessary to use the alcohol method for sugars. When this method is used the residue from the petroleum ether extraction should be transferred to a 300 cc. flask with 50 per cent alcohol.

#### SUCROSE.

Calculate from the difference between the reducing sugar after inversion and before inversion.

#### TOTAL ACID.

Titrate 10 grams of the sample in 400-500 cc. of recently boiled and cooled water with standard alkali, using phenolphthalein as an indicator. Calculate as acetic acid.

#### OIL.

Determine by the Roese-Gottlieb method<sup>2</sup> on 2 grams of the sample using 2 cc. of concentrated ammonium hydroxide, 10 cc. of alcohol, and enough water to fill the tube to just below the outlet, and making at least four extractions. Dry the oil in a vacuum oven at 70°C. to constant weight. (The residue remaining in the tube may be used for testing for turmeric.)

#### IDENTIFICATION OF THE OIL.

The residue obtained by evaporating the petroleum ether used to extract the oil in the sugar determination can be used for this purpose and the identity of the oil established by the usual chemical and physical tests.

#### LECITHIN-PHOSPHORIC ACID ( $P_2O_5$ ).

Transfer the residue obtained in the determination of total solids to an extraction thimble adding also the lead dish, which has been cut into pieces for this purpose. Extract with absolute alcohol in an extractor of the siphon type in which the vapor heats the contents of the siphon thimble. In case this is not available and a Soxhlet extractor is used, it should be wrapped with paper to raise the temperature. After 10 hours' extraction saponify the extract with alcoholic potassium hydroxide (for each gram of fat present use 5 cc. of a solution containing 8 grams of KOH per 100 cc.) in a beaker or large platinum dish. Evaporate to dryness and ignite.

Evaporate to dryness on a water bath and char over asbestos. Treat the charred mass with dilute nitric acid, filter, and wash with water. Return the residue with the paper to the platinum dish and burn to a white ash. Treat again with nitric acid, filter, and wash, uniting the filtrates. In the combined liquid, determine phosphoric acid by the usual method.

<sup>1</sup> Assoc. Official Agr. Chemists, *Methods*, 1920, 78.

<sup>2</sup> *Ibid.*, 94

<sup>3</sup> *Ibid.*, 227

Juckenack<sup>1</sup> gives the following average values for average-sized eggs:

	WEIGHT OF MATERIAL	DRY SUBSTANCE	TOTAL P <sub>2</sub> O <sub>5</sub>	LECITHIN P <sub>2</sub> O <sub>5</sub>
	grams	grams	gram	gram
Yolk . . . . .	16	7.835	0.2046	0.1316
White . . . . .	31	4.540	0.0097	0.0000
Whole egg . . . . .	47	12.375	0.2143	0.1316

By using these figures the approximate egg content of a dressing can be calculated from the lecithin P<sub>2</sub>O<sub>5</sub> content.

#### PREPARED MUSTARD.

The present official and tentative A. O. A. C. methods include, and for a number of years have included, a tentative method for crude fiber in prepared mustard that is by no means entirely satisfactory. Accordingly, during the year 1921, a new method was submitted for this determination. During that same year it was proposed to change the general method for crude fiber. It was the attitude of the referee that his recommendation for adoption of the details submitted by him concerned only the preliminary treatment of the sample, and that the actual fiber determination should be made according to the method which may be official at the time. Any change, at any time, in the official general method should automatically apply to the method for prepared mustard. However, the details proposed were not adopted at that time in view of the proposed changes in the general method.

During the following year, 1922, the referee gained the impression that the question of methods for crude fiber in prepared mustard should be given consideration by the Referee for Crude Fiber. This error was corrected too late in the season for any actual collaborative work. In view of all the circumstances it was again recommended that the method be made tentatively official, without such additional investigation. There was introduced, however, a slight change in the details, in that the use of a 1000 cc. flask was directed instead of a 500 cc. flask. This was due to an error.

The method was adopted, so that two tentative methods are now available. The committee directed that during the present year these two methods be given comparative study, with a view, it is assumed, to the elimination of one of them.

In connection with this year's work, careful consideration was again given to the results obtained in 1921<sup>2</sup>. It will be noted that the results reported on the A. O. A. C. tentative method were quite unsatisfactory—so much so, in fact, that the referee considered it desirable to replace it by a modification devised by Hilts and Hertwig. As previously stated, their method was tentatively adopted during the following year, but the existing tentative method was retained.

<sup>1</sup> *Z. Nahr. Genussm.*, 1900, 3: 11.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 95.

It will be noted that the old tentative method requires that the mustard—fat and all—be boiled with the respective acid and alkaline solutions, but that the crude fiber, after drying, be washed with ether to remove the fat. The newer tentative details require removal of the fat before boiling. However, there was also submitted to collaborators during 1921 a slight modification of the old tentative method, which required that after boiling the crude fiber be washed successively with water, alcohol, and ether, and then dried. This procedure gave better results, but they were not quite so satisfactory as those obtained by the method of Hilts and Hertwig. Furthermore, in connection with spices and other foods and feeding stuffs, extraction with ether is accomplished before boiling, and this seems to afford an additional reason for a similar procedure for mustard.

Taking everything into consideration, it was believed advisable, during the present year, to submit to collaborators the new tentative method, and also the old tentative method, modified so as to require removal of the fat after boiling but before drying.

One sample only was submitted to collaborators—a commercial sample of prepared mustard. Collaborators were requested to apply the two methods indicated.

#### COLLABORATORS' RESULTS.

It is regretted that only three collaborators reported on this sample. However, their results are interesting and conclusive.

TABLE 2.  
*Results on crude fiber in prepared mustard.*

DETERMINATIONS	BERRY	BORNHANN	GREENLEAF
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Total solids	14.28	14.41	14.46
Crude fiber—			
Method No. 1* . . . .	1.69 1.60	1.21 1.23	1.33 1.25
Method No. 2† . . . .	1.26 1.21	1.18 1.15	1.23 1.18

\* *J. Assoc. Official Agr. Chemists*, 1922, 6: 94, Method II

† *Ibid.*, Method III.

These results are in accord with those obtained in 1921 and show that the new tentative method gives satisfactory results, distinctly more so than the old method, even with the necessary modification that was applied by collaborators this year.



## RECOMMENDATIONS.

As a result of this year's work, in conjunction with the results previously obtained, it is recommended—

- (1) That the present official method<sup>1</sup> be replaced by the following:

*Method No. 2.*

(Proposed by Hilts and Hertwig.)

Weigh 10 grams of the sample and transfer to an 8 ounce nursing bottle with 50 cc. of strong alcohol, stopper, and shake vigorously. Add 40 cc. of ethyl ether, shake, and let stand about 5 minutes with occasional shaking. Centrifugalize and decant off the alcohol-ether mixture. Treat twice more with 40 cc. portions of ether, shaking, centrifugalizing, and decanting as before. Rest the bottle on its side for a short time, without heat, to allow the ether largely to evaporate. Transfer the material to a 500 cc. Erlenmeyer flask, using 200 cc. of boiling hot dilute sulfuric acid and proceed as directed in VII, 66.

If preferred, the sample may be treated with the alcohol and ether in a small beaker, transferred to a hardened 11 cm. filter paper, washed several times with ether, and finally transferred to a 500 cc. Erlenmeyer flask with 200 cc. of boiling hot dilute sulfuric acid.

- (2) That appropriate action be taken by the association, whereby any future change in the general method for crude fiber will automatically apply to prepared mustard.

REPORT ON THE MICROSCOPICAL EXAMINATION OF  
CACAO PRODUCTS.

By V. A. PEASE (Bureau of Chemistry, Washington, D. C.), *Referee*.

Although a large amount of work has been done during the past year to perfect the method already tentatively adopted by this association for the determination of cacao shells in cacao products<sup>2</sup>, most of this has been preliminary and has not reached a point where it can be presented in the form of a report. This work includes:

- a. Experimental work by the referee;
- b. Analysis of a series of samples by six analysts in branch laboratories of the Bureau of Chemistry, in collaboration with the referee; and
- c. The preparation of material for standards containing known amounts of shell, to be made up under factory conditions.

The experimental and collaborative work shows conclusively the necessity for having standards made up in the factory, but the details of the work have not been completed.

The preparation of the material for standards involves a vast amount of preliminary work, as the roasted and cracked cacao beans must be absolutely freed from shell before known amounts of shell can be added

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 98.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 99.

to the nibs to make mixtures of definite percentage composition. More than a ton of material has been carefully cleaned and recleaned under the supervision of the referee and is now ready for the process of manufacture.

The new standards will probably be ready early in the year, and it is hoped that their careful study, both from the chemical and the microscopical point of view, will clear up several points in the analysis of cacao products that are still rather vague.

In connection with the samples that were sent out to collaborators, the referee suggested that a modified chloral hydrate solution be given a trial as a mounting medium. This solution was proposed in 1921 by Raymond Hertwig, at that time of the San Francisco Station and now of the Food Control Laboratory of the Bureau of Chemistry. It has been used by two analysts in the Microchemical Laboratory for several months, in general work as well as in the examination of cacao products, and seems to be a note-worthy improvement over the standard 1 : 1 chloral hydrate clearing solution, especially in quantitative work where it is necessary to hold a slide for some time. The formula is as follows:

HERTWIG'S SOLUTION:

25 cc. dilute hydrochloric acid (1 + 8);  
10 cc. glycerol; and  
45 grams chloral hydrate.

The idea of using glycerol with chloral hydrate as a clearing solution is not new. Kraemer<sup>1</sup> mentions a chloral-glycerin solution made up of equal parts of glycerin and water saturated with chloral. Winton<sup>2</sup> notes that a chloral hydrate solution gradually develops acidity on standing and recommends that a freshly made solution be acidified at once by the addition of hydrochloric acid. The formula suggested by Hertwig, however, is the first instance, so far as the referee can ascertain, in which chloral hydrate, glycerol, and hydrochloric acid have been combined to form a clearing and mounting medium.

Care must be taken to use just enough of the solution to fill in under the cover glass, as the glycerol becomes more fluid on warming, and if too much is used fragments of tissue are carried out beyond the edge of the cover glass. The advantages of the solution are (1) that it seems to do away with the drying out of the mount while counting; (2) it prevents the formation, under the edges of the cover glass, of crystals which obscure part of the mount; and (3) it quickens the procedure—that is, the mount can be made up, warmed for about 20 minutes, and counted after standing for only half or three-quarters of an hour. The disadvantages are the spreading of the mount, if care is not used, and

<sup>1</sup> *Text-book of Botany and Pharmacognosy*. 4th ed., 1910, p. 802

<sup>2</sup> *The Microscopy of Vegetable Foods*. 2nd ed., 1916, p. 185.

the formation of minute crystals of ammonium chloride on the surface of the cover glass, if the work must be done in a chemical laboratory where ammonia fumes are present in the atmosphere.

All the collaborating analysts reported that they were much pleased with the modified solution. Both the advantages and the disadvantages were discussed, but it seems to be the general opinion that the good points far outweigh the bad, and that the use of Hertwig's solution will add to the efficiency of the method.

#### RECOMMENDATIONS.

It is therefore recommended—

(1) That the work on the perfecting of the microscopical method for the detection of cacao shells in cacao products be continued.

(2) That the use of Hertwig's solution as a mounting and clearing medium be included in the description of the method.

#### REPORT ON CHEMICAL EXAMINATION OF CACAO PRODUCTS—EXPERIMENTS ON CRUDE FIBER CONTENT.

By E. R. MILLER (Food and Drug Inspection Station, New York, N. Y.),  
*Associate Referee.*

Among the chemical determinations in cacao products upon which further investigation and study were desired was crude fiber. The investigation of this substance, therefore, was chosen as the subject of this report.

A preliminary survey of the existing methods, the official<sup>1</sup> and the Bidwell-Bopst<sup>2</sup>, brought out some objections. The official method requires that acid filtrations taking more than 5 minutes be discarded and that a new assay be started. The main objection to the Bidwell-Bopst method is the use of asbestos. Correspondents also argued that one gram of asbestos was too bulky, that there was a loss in weight due to ignition, or that the asbestos induced bumping.

A sample, bearing the following declaration of the manufacturer: "A Chocolate Compound containing Pure Cacao Nibs 60%, Tailings 35%, and Cacao Butter substitute 5%", was sent out to fourteen collaborators, together with copies of three different methods, the official, the Bidwell-Bopst, and the following modification of the official method:

*Proposed method for the determination of crude fiber in cacao products.*

#### SPECIAL REAGENTS

(a) Dilute sulfuric acid.—1.25 per cent determined by titration.

(b) Dilute sodium hydroxide.—1.25 per cent determined by titration. Should be practically free from carbonate.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 97.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 339.

## DETERMINATION.

Extract a dry 2 gram sample of chocolate liquor (bitter) or cocoa (5 gram sample of sweet chocolate, milk chocolate, or sweet cocoa) as follows: Solvent, anhydrous ethyl ether. Extraction carried out in a Knorr or Soxhlet apparatus of the continuous type. If a Soxhlet is used, the seal at the bottom should be punctured to make a straight continuous extraction tube. The cacao material may be contained either in an alundum crucible (Norton Alundum No. 5204 R.A. 360, E.&A. No. 2382) or in a filter paper (C. S. & S. No. 575 hard 12½ cm.) fitted into a Knorr tube. The extraction is carried out for 4 or 5 hours as in the official method for fat. After the fat has been extracted change the receiver and continue the extraction, using as a solvent 95 per cent ethyl alcohol (to remove the cacao red) for 7 hours. Dry and proceed as follows:

To the powdered residue contained in a 500 cc. Erlenmeyer flask add 200 cc. of the boiling dilute sulfuric acid solution, connect the flask with a reflux condenser and a stream of air from the blast (to prevent excess frothing), boil at once, and continue boiling gently for exactly 30 minutes. Filter through filter paper (Whatman No. 12 folded 18½ cm.). Reject if filtration requires more than 5 minutes. Wash free of acid with boiling water. Rinse the material back into the flask by means of a short-stemmed funnel with boiling dilute sodium hydroxide solution. Connect with the condenser and stream of air, as directed previously, bring immediately to a boil, and continue boiling gently exactly 30 minutes. Filter immediately through the filter paper, washing free of alkali with boiling water. Use as indicator. Transfer to a platinum or silica dish (E. & A. 2648 No. 5) by means of a stream of 95 per cent ethyl alcohol. Evaporate the alcohol and dry at the temperature of boiling water to constant weight. Weighing should be rapid on account of the hygroscopic nature of crude fiber (cellulose). Incinerate and weigh again. The loss in weight is considered to be crude fiber.

The results shown in the table were reported by the referee and the following collaborators:

M. L. Offutt, Food and Drug Inspection Station, New York, N. Y.

Doris McIntire, Food and Drug Inspection Station, San Francisco, Calif.

C. A. Roach, Transportation Building, Chicago, Ill.

Leonard Feldstein, Food and Drug Inspection Station, Denver, Colo.

C. A. Greenleaf, Food and Drug Inspection Station, Cincinnati, O.

S. C. Rowe, Food and Drug Inspection Station, Philadelphia, Pa.

The cause which led to the particular modification suggested was the number of assays that had to be rejected on account of the 5 minute time limit on acid filtration. One sample in particular that defied determination by the present official method led the referee to examine more closely the cause of the protracted filtration. Ruling out the variability due to the use of different filter paper by adopting one variety, observation led to the notation that this particular sample contained relatively large quantities of an alcohol-soluble material whose behavior would indicate something of the nature of a colloid. Treatment of the ether-extracted residue of this sample with 95 per cent ethyl alcohol removed the troublesome cause of slow filtration and reduced the time for acid filtration from 30 to 4 minutes.

*Collaborative results on determination of crude fiber in cacao products.*

## BIDWELL-BOPST METHOD.

COLLABORATOR	CRUDE FIBER— SAMPLE BASIS			AVER- AGE	DIFFER- ENCE	AVER- AGE ACID FILTRA- TION	ALKALI FILTRA- TION	FIBER	
								Moisture- fat free basis	Differ- ence
	<i>per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>minutes</i>	<i>minutes</i>	<i>per cent</i>	<i>per cent</i>
Rowe	4.95	4.78	4.98	4.88	0.00	5.0	3.0	8.66	0.00
McIntire	5.00	4.90		4.95	0.07	3.0	5.0	8.79	0.13
Roach	4.90	5.00		4.98	0.10	4.0	4.0	8.84	0.18
Offutt	5.13	5.15		5.14	0.26	1.3	1.5	9.13	0.47
Miller	5.19	5.17	5.15	5.17	0.29	1.3	1.8	9.18	0.52
Greenleaf	5.19	5.18		5.19	0.31	0.8	1.5	9.21	0.55
Feldstein*	5.77	5.88		5.81	0.93	5.5	1.6	10.32	1.66

## MODIFIED METHOD.

Miller	4.99	4.98	4.99	4.99	0.00	6.0	2.8	8.86	0.00
McIntire	5.06	5.05		5.06	0.07	3.0	5.0	8.98	0.12
Offutt	5.10	5.03		5.07	0.08	3.8	3.5	9.00	0.14
Roach†	5.15	5.00		5.08	0.09	3.0	2.0	9.01	0.15
Rowe	5.16	5.09		5.13	0.14	4.5	3.5	9.11	0.25
Greenleaf	5.28	5.23		5.26	0.27	2.8	1.0	9.33	0.47
Feldstein*	5.47	5.64		5.56	0.57	4.1	2.6	9.86	1.00

## OFFICIAL METHOD.

McIntire†	4.60	4.45		4.53	0.00	3.0	5.0	8.03	0.00
Roach	4.95	4.90		4.93	0.40	2.0	2.0	8.74	0.71
Greenleaf	4.96	4.90		4.93	0.40	0.5	0.5	8.75	0.72
Rowe	5.13	5.01		5.07	0.54	3.3	3.0	9.00	0.97
Miller	5.15	5.11	5.06	5.11	0.58	20.6	3.2	9.06	1.03
Offutt	5.20	5.25		5.23	0.70	5.0	6.0	9.29	1.26
Feldstein*	5.23	5.29		5.26	0.73	5.3	1.6	9.34	1.31

\* Denver—5280 feet elevation.

† Linen.

It was also noted that the acid solution became cloudy on cooling, showing that some of the acid-soluble material is insoluble at temperatures slightly below boiling. This is interesting since it offers an explanation for the high results obtained by the Denver collaborator. Owing to the elevation, a boiling solution would not be so hot there as a boiling solution at sea level; consequently this material would not be dissolved, and results would be higher than those obtained at sea level.

In the table variations may be noted in each of the three methods. If the high results obtained by the Denver collaborator are disregarded, assuming the explanation of difference in barometric pressure to be correct, it will be observed that the smallest variation occurs in the modified method, if the first results are taken for basis of comparison, namely, 0.27 per cent (0.00 to +0.27). The Bidwell-Bopst method comes next with 0.31 per cent (−0.00 to +0.31), and the official is highest with 0.70

per cent ( $-0.00$  to  $+0.70$ ). This variation may be partly accounted for by individual variations in the method of application. At best, any crude fiber method is and must be empirical, and therefore exact following of the method is mandatory.

In the personal application of the modified method the writer has found results to be lower than those obtained by the other two methods. It may be that the alcohol removes some inhibitory agent or binder that allows the acid solution a much fuller action. If an alundum thimble or crucible is used for the fat extraction, a nicely divided paste can be worked up with a policeman during the transfer to the Erlenmeyer flask. This opens up the following questions: What is crude fiber? Is it to be defined as the result obtained by manipulating a few chemicals in a certain limited way or is a more definite chemical compound, such as an impure lignone-cellulose complex sought?

The sample used is known to contain a foreign fat. The following results of a 5 hour fat extraction carried out in the New York laboratory show the necessity for complete fat extraction.

MODIFIED METHOD	OFFICIAL METHOD	BIDWELL-BOPST METHOD
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
5.88	5.95	5.88
5.85	5.98	5.90

Bidwell and Bopst have found that incomplete fat extraction increases the reported crude fiber content. It is important, therefore, when a foreign fat is present or when large quantities of fat are present, as in a case of chocolate liquor, to increase the time of fat extraction beyond the 5 hour period.

#### COMMENTS BY COLLABORATORS.

*M. L. Offutt.*—The Bidwell-Bopst method appears to be the most rapid and requires less manipulation than the modified method. The modified is far superior to the official method and if an alundum crucible or thimble is used instead of filter paper I believe it would be satisfactory. Preference is given the Bidwell-Bopst and modified methods.

*C. A. Roach.*—Modified Method. Used air condensers. In official and Bidwell-Bopst methods fat was extracted by centrifuge; filtration through linen. Preference is given modified and official methods as use of the Liebig condenser is somewhat troublesome when the flasks must be shaken. Also, 1 gram of asbestos makes too bulky a mass to put into a Gooch crucible.

*Leonard Feldstein.*—In regard to the modified method I would suggest that filtration be made on linen. On many occasions we have found that Whatman filter paper is very soft and frequently is torn in washing. I wish to call your attention to the fact that Denver is 5280 feet above sea level, and that this altitude causes a lowering of the boiling point of water to  $94^{\circ}\text{C}$ . This would indicate that my results would be higher than those obtained by collaborators working at sea level.

*C. A. Greenleaf.*—In the modified method, the fiber showed a tendency to decrepitate on drying. Extractions were carried out in a Johnson apparatus, using alundum thimbles.

*S. C. Rowe.*—The essential difference between the Bidwell-Bopst method and the other methods which checked pretty close is the addition of approximately 1 gram of asbestos. This addition showed up the filtrations and made transfers more cumbersome. In the modified method I noted the removal of the cacao red decreased the frothing during the acid and alkali boilings.

#### DISCUSSION.

In view of the results obtained by the various collaborators and the effect of even small differences in the crude fiber content when computed to the fat-moisture-and-sugar-free basis, the referee recommends the further investigation of this problem. At present some authentic samples are being made up under factory conditions, and it is hoped that analyses of these samples will throw much light on the influence of or changes due to manufacture. It is also recommended that investigations be carried on by a so-called "cold process" for fiber determination, whereby the high results obtained at great elevations above sea level can be avoided, and whereby determinations carried on in different sections of our country will be independent of barometric pressure.

#### SUMMARY.

A survey of existing methods shows a desirability for further investigation of crude fiber determination of cacao products

Slow filtration is due to separation of cacao solids insoluble in temperatures slightly lower than the boiling point of the solution. Troublesome causes were removed by the treatment of ether extracted residue with 95 per cent ethyl alcohol.

Any crude fiber method is empirical. A strict following of method is mandatory.

Thorough fat extraction is necessary.

#### REPORT ON TEA<sup>1</sup>.

By **R. E. ANDREW** (Agricultural Experiment Station, New Haven, Conn.), *Referee*.

No cooperative work on this subject has been conducted during the past year.

It is recommended—

(1) That the proposed method, as described in the referee's report for 1922<sup>2</sup>, be adopted as an official method for the determination of water extract in tea.

(2) That suggestions for further study on the subject of tea be left to the next referee.

<sup>1</sup> Presented by A. J. Patten.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 155.

No report on methods for the examination of cacao butter was made by the referee.

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The convention adjourned at 3 o'clock, Wednesday, November 21st. The proceedings for Friday morning and afternoon were published in Vol. VII, No. 3.

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#### COMMITTEE ON BIBLIOGRAPHY.

During the discussion on the revision and publication of the *Book of Methods* R. E. Doolittle suggested that a committee be appointed to study the subject of bibliographies and, if considered necessary, to arrange for complete bibliographies for all the methods.

A motion was made and carried that such a committee be appointed. Later the following committee was named:

W. W. Skinner (Bureau of Chemistry, Washington, D. C.), Chairman.

G. S. Fraps,

H. D. Haskins,

F. P. Veitch,

W. W. Randall.



# CONTRIBUTED PAPERS.

## THE ANALYSIS OF PHOSPHATE ROCK<sup>1</sup>.

By G. E. F. LUNDELL, Chemist, and J. I. HOFFMAN, Associate Chemist  
(Bureau of Standards, Department of Commerce, Washington, D. C.).

### I. INTRODUCTION.

In the analysis of its standard phosphate rock sample No. 56, the Bureau of Standards enjoyed the cooperation of the following companies and chemists:

Armour & Company, Chicago, Ill. Paul Rudnick, Chief Chemist.  
Virginia-Carolina Chemical Co., Richmond, Va. F. B. Carpenter, Chief Chemist.  
Wiley & Company, Inc., Baltimore, Md.  
Shuey & Company, Savannah, Ga.  
McCandless Laboratory, Atlanta, Ga.  
Swift & Company, Chicago, Ill. W. D. Richardson, Chief Chemist  
The American Agricultural Chemical Co., Carteret, N. J. J. E. Breckenridge, Chief Chemist.  
F. S. Royster Guano Co., Norfolk, Va. E. W. Magruder, Chief Chemist.

The work has brought out a number of interesting facts concerning the performance of the old methods for the determination of moisture, phosphoric acid, and "soluble iron and alumina"<sup>2</sup> and has resulted in the discovery of desirable changes in these methods as well as in the development of certain new methods.

The results seem sufficiently interesting to bring to the attention of the readers of this Journal. Although special attention is paid to the usual determinations listed above, methods for the less commonly determined total ferric oxide, alumina, and lime are also included because they are of general interest.

### II. GENERAL DISCUSSION.

#### A. MOISTURE.

In careful work all analyses must be made on the basis of the finely ground sample (80 mesh or finer), which has been dried at 105°C. The moisture content of air-dried samples, like the Bureau of Standards sample No. 56, may vary appreciably. For example, analyses of this sample in Washington, D. C., showed 0.6 per cent of moisture in February as against 0.9 per cent in August, a difference of 0.3 per cent or 0.1 per cent of  $P_2O_5$  on an air-dry basis. Determinations of moisture depend upon various factors, such as the temperature and ventilation of the oven, duration of heating, depth of the layer of sample, and secondary changes

<sup>1</sup> Published by permission of the Director, Bureau of Standards, Department of Commerce.

<sup>2</sup> This expression denotes soluble iron and aluminium expressed as ferric and aluminium oxides and is kept throughout the paper because of its common use in the fertilizer industry.

such as oxidation. The variations in temperature in different parts of ordinary ovens are too well known to need comment. The most desirable drying temperature is 105°C. as this promotes rapid drying and excludes the use of the uncertain temperatures of water or steam ovens. When portions of sample No. 56 were dried in a well-ventilated oven for one hour, 20 per cent less moisture was obtained at 100°C. than at 105°C. On the other hand, the results at 110°–115°C. were practically the same as at 105°C. Ventilation of the oven by proper vents is necessary, and the passage of a current of dry air is desirable. One hour is sufficient for the drying of samples at 105°C. in well-ventilated ovens provided the depth of the sample is not over 0.5 cm. Tests showed that samples of this depth never retained over 5 per cent of their moisture, while samples of 0.8–1.0 cm. layer often retained as much as 20 per cent. Secondary changes, such as oxidation, which may take place during the heating of the sample, are disregarded in work of this kind.

## B. PHOSPHORIC ACID.

### (a) SOLUTION TREATMENT.

Treatment of the rock with aqua regia followed by evaporation to sirupy consistency, as prescribed by the Fertilizer Division of the American Chemical Society<sup>1</sup> and in the official methods of the Association of Official Agricultural Chemists<sup>2</sup>, is quite satisfactory for the solution of phosphate rock. In sample No. 56 only 0.02–0.03 per cent of  $P_2O_5$  remains insoluble after this treatment. It is not certain, however, that all the fluorine content of the rock will be expelled. Fluorine tends to retard the precipitation of phosphomolybdate and to cause the formation of a precipitate that is more soluble in washing solutions. The authors have found that boric acid overcomes these difficulties<sup>3</sup>, and as there is no trouble and practically no expense connected with its use, an addition of boric acid after the preliminary attack is recommended.

### (b) PRECIPITATION AND FILTRATION OF AMMONIUM PHOSPHOMOLYBDATE.

All methods for the determination of phosphoric acid in phosphate rock call for its preliminary separation as ammonium phosphomolybdate. This precipitation succeeds best in a warm solution (40°–65°C.) having a volume of from 100–200 cc. and containing 5–10 per cent by volume of nitric acid (sp. gr. 1.42), 5–15 per cent of ammonium nitrate, and a 25–50 cc. excess of the molybdate precipitant. If the alkalimetric method is to follow, the solution must not be heated after the molybdate reagent is added, and before this addition it should be sufficiently hot to assure a final temperature of approximately 40°C. As the heat of neutralization

<sup>1</sup> *J. Ind. Eng. Chem.*, 1915, 7: 446

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 2 (2)

<sup>3</sup> *J. Ind. Eng. Chem.*, 1923, 15: 44

of nitric acid provides a simple way of warming the solution, at the Bureau of Standards an excess of nitric acid is taken at the start, and some of this excess is afterwards neutralized by ammonia to give the necessary temperature and concentration of ammonium nitrate. Satisfactory precipitations for the alkalimetric method can be made at 20°C., but these are somewhat slower unless continuous shaking is used. When the gravimetric method is to follow, changes in the  $\text{MoO}_3$  content of the phosphomolybdate are of no consequence, and precipitations can be made at higher temperatures; in such runs it is desirable to start with a warm solution and then to allow it to cool and stand overnight.

Aside from fluorine, which has already been mentioned, phosphate rock is not likely to contain substances that seriously retard or contaminate the phosphomolybdate precipitate<sup>1</sup>. Certain of these may be introduced by the analyst, however, notably hydrochloric or sulfuric acid. Both should be avoided if possible, and sulfuric acid should not be used in umpire analyses.

The filtering medium for phosphomolybdate precipitates is largely a matter of choice; some analysts prefer filter paper and some pulp or asbestos with suction. Suction is desirable when but a few solutions are to be filtered; when much work is done, good papers and funnels take care of the solutions as fast as they can be handled.

#### (c) GRAVIMETRIC METHODS.

In the analysis of phosphate rock practically all gravimetric methods call for solution of the phosphomolybdate precipitate and reprecipitation of phosphorus as  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ . Other gravimetric methods, such as weighing the phosphomolybdate or precipitating the molybdenum in it as  $\text{PbMoO}_4$ , need not be considered, because they are no more accurate than the more rapid alkalimetric procedure.

Single precipitations as magnesium ammonium phosphate can never give correct results in the analysis of phosphate rock except through fortunate balancing of errors, for the precipitate always contains molybdenum and it is rarely, if ever, of ideal composition.

The contamination of the precipitate by molybdenum is not surprising in view of the 3 grams or more of  $\text{MoO}_3$  that is present when the precipitate is formed. Practically the same quantity, from 1–2 mg. of  $\text{MoO}_3$ , is carried down, regardless of whether the magnesia mixture is added to an acid, a neutral, or an ammoniacal solution of the phosphomolybdate. Some of the molybdenum is volatilized when the precipitate is ignited—more of it when the precipitate is caught on paper and ignited in platinum than when it is ignited in porcelain Gooch crucibles. For example, ammoniacal solutions of phosphomolybdate precipitates were treated with

<sup>1</sup> For discussion of such substances, see an article by the authors, *J. Ind. Eng. Chem.*, 1923, 15: 44.

5 cc. of hydrochloric acid in excess in some cases, with 5 cc. of ammonium hydroxide in excess in others, and were made just neutral in the remainder. Magnesia mixture in 15 cc. excess was then added at the same fairly rapid speed to each solution; the solutions were made distinctly ammoniacal and allowed to stand. The precipitates were caught on paper and ignited in platinum crucibles. The acid series gave 0.2393, 0.2388, and 0.2394 gram of  $\text{Mg}_2\text{P}_2\text{O}_7$ ; the ammoniacal series gave 0.2393, 0.2387, and 0.2394 gram; while the "neutral" series gave 0.2392, 0.2391, and 0.2395 gram. The correct value was 0.2370 gram. The high results were ascribed to excess magnesia, as the molybdenum content of each of the precipitates was practically the same and averaged 0.4 mg. of  $\text{MoO}_3$ . In experiments with another standard solution, four precipitates were obtained as in the "acid" series. Two that were caught in paper and ignited in platinum weighed 0.2113 and 0.2114 gram, while the other two caught in porcelain Gooch crucibles and ignited over blast lamps weighed 0.2140 and 0.2126 gram. When the latter were transferred from the asbestos pads to platinum crucibles and reheated, the weights dropped to 0.2123 and 0.2109 gram.

The composition of the precipitate depends on the ratio of phosphate to magnesia at the moment the precipitate is formed. Once formed, its composition is not materially changed by long standing at room temperature. If an excess of magnesia is present while the precipitate is forming, high results are the rule, possibly because of the formation of some  $\text{Mg}_3(\text{PO}_4)_2$ . For example, aliquot portions of a phosphate solution gave 0.2370 and 0.2370 gram of  $\text{Mg}_2\text{P}_2\text{O}_7$  with 2 cc. of magnesia mixture in excess and 0.2397 and 0.2394 with 20 cc. in excess at the time of precipitation. If the mixture is added to a neutral or ammoniacal solution so slowly that phosphate is still in marked excess when precipitation starts, low results are the rule, perhaps owing to the precipitation of some  $\text{Mg}(\text{NH}_4)_4(\text{PO}_4)_2$ . For example, 0.2308 and 0.2297 gram of  $\text{Mg}_2\text{P}_2\text{O}_7$  was obtained when magnesia mixture was added at the rate of 1 cc. per minute to cold ammoniacal portions of a standard solution of phosphate, 0.2330 gram when it was added at 2 cc. per minute, and 0.2370 gram when it was added all at once. In further tests with aliquot portions of the same solution the phosphomolybdate precipitates were dissolved in ammonia; the solutions were made just neutral to litmus, treated with magnesia mixture, allowed to stand for 12 hours, and filtered in porcelain Gooch crucibles; and the precipitates were ignited over blast lamps. As compared with the true value, 0.2370 gram of  $\text{Mg}_2\text{P}_2\text{O}_7$ , the addition of magnesia mixture at a rate of 1 cc. per minute gave 0.2356 gram in a cold solution and 0.2347 gram in one at  $40^\circ\text{C}$ . With rapid addition of the mixture the results were 0.2400 and 0.2379 gram, respectively. Precipitates that are deficient in magnesia should be avoided even when double precipitations are contemplated for they are more soluble in ammoniacal wash solutions.

It is obvious from the foregoing results that double precipitations of magnesium ammonium phosphate are absolutely necessary in order to avoid an improper excess of magnesia mixture and contamination by molybdenum. The loss of phosphorus through repeated precipitation is negligible. In analyses of six aliquot portions of a standard solution of phosphate (without preliminary precipitation as the phosphomolybdate), 0.2367 and 0.2368 gram of  $\text{Mg}_2\text{P}_2\text{O}_7$  was obtained by single, 0.2370 and 0.2368 gram by double, and 0.2366 and 0.2368 gram by triple precipitations. An average of less than 0.1 mg. of  $\text{P}_2\text{O}_5$  was lost in each precipitation as was shown by careful recovery of the phosphorus in the combined filtrates and washings from ten of the twelve precipitations.

It is also apparent that the results of single precipitations will be improved and rendered more uniform if the magnesia mixture is added in not more than a 2-3 cc. excess to an acid solution of the phosphomolybdate and if the precipitate is caught and ignited in paper.

In the analysis of sample No. 56, the results obtained by the cooperating analysts showed an extreme variation of 1.07 per cent and an average deviation from the certificate value of 0.40 per cent when a single precipitation (as in the A. O. A. C. method<sup>1</sup> for fertilizers) was made. At the Bureau of Standards the results showed an extreme variation of 0.31 per cent and an average deviation of 0.13 per cent. When the first precipitate was dissolved and reprecipitated, after the addition of a small quantity of magnesia mixture, the cooperators' results showed an extreme variation of 0.84 per cent and an average deviation of 0.34 per cent, while those of the Bureau analysts gave an extreme variation of 0.1 per cent and an average deviation of 0.023 per cent. The writers believe that analyses by the double precipitation method should be accurate to  $\pm 0.05$  per cent.

The following additional notes dealing with the gravimetric method may be of interest.

1. Moderate quantities of calcium do not interfere in the precipitation of phosphate by magnesia mixture. In tests made by the writers, 0.01 gram of  $\text{CaO}$  added to either the solution or the magnesia mixture caused no contamination of the precipitate in single precipitations, and 0.1 gram of  $\text{CaO}$  added to the solution caused no difficulty in double precipitations.

2. Some phosphoric acid may be lost as ferric phosphate, titanium phosphate, etc., when the phosphomolybdate is dissolved in ammonia and then filtered as in the A. O. A. C. method for fertilizers. When titanium is present in the rock the loss may be appreciable, as occasionally over half of the titanium comes down in the yellow precipitate. Addition of citric acid to the ammoniacal solvent is recommended, since this acid aids the solution of these compounds and afterwards retards their coprecipitation with magnesium ammonium phosphate. To render the

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 2.

solution of ferric phosphate more certain, the paper should also be washed with a little hot dilute hydrochloric acid (1:20).

3. The use of an ammoniacal magnesia mixture is not necessary. Neutral or very slightly acid solutions serve just as well and do not attack the reagent bottle.

4. When the magnesium-ammonium phosphate precipitate is dissolved and reprecipitated, a little magnesia mixture must be added before the second precipitation is made. Reasonable variations in the quantity of ammonium chloride are permissible, as much as 15 grams per 100 cc. being without ill effect.

5. A washing solution containing 5 per cent by volume of ammonium hydroxide (sp. gr. 0.90) suffices for anything but the most exacting work.

6. If the precipitate is burned in paper, the ignition should not be hurried at first, but it should be carried on under good oxidizing conditions at a low temperature until the carbon is gone.

After the final weighing the precipitate should be examined for gray or black areas inside the ignited lump.

#### (d) ALKALIMETRIC METHOD.

The alkalimetric method for phosphorus is a valuable routine method and is capable of giving excellent results in the hands of analysts who are familiar with it. Rigid adherence to details are necessary in any modification of the method, and the 23 NaOH:1 P ratio can be used only when certain conditions as to temperature of precipitation and manner of washing the precipitate are met. It is interesting to note that several of the cooperating analysts stated that it is necessary to modify the A. O. A. C. methods for the alkalimetric determination of phosphorus by the use of empirical titres in order to obtain correct results.

Results obtained at the Bureau of Standards by the use of the alkalimetric method, which is recommended in (III B d), showed an extreme variation of 0.61 per cent and an average deviation from the certificate value of 0.13 per cent. An accuracy of  $\pm 0.15$  per cent can be expected by the use of this method under exceptional conditions, while  $\pm 0.3$  per cent is a fair estimate of its accuracy in good routine work.

The following notes dealing with the alkalimetric method may be of interest:

1. No alkalimetric method should call for heating of the solution after the molybdate reagent has been added. Precipitation at 40°C. in a solution that has been heated by partial neutralization of nitric acid is rapid and satisfactory. Precipitation at room temperature is slow unless the solution is shaken continuously.

2. Results obtained by precipitation at 60°C. or higher are often inconsistent if an empirical titre is employed and are always high if the 23:1 ratio is used as in the A. O. A. C. method (a).

3. The manner of washing the phosphomolybdate precipitate is very important in alkalimetric methods. Washing with water as in the A. O. A. C. method (b) leads to high results, while the same manner of washing the differently formed precipitate obtained in the A. O. A. C. method (c) gives quite satisfactory results. Washing with a 5 per cent solution of ammonium nitrate gives high results, chiefly because of the effect of the residual ammonium nitrate upon the phenolphthalein end point. The commonly used 1 per cent solution of potassium nitrate has proved quite satisfactory in the hands of the writers, in spite of its slight solvent action on the precipitate. This is so because a fairly well defined washing procedure is used, and the loss of phosphomolybdate tends to compensate for the extra amount of sodium hydroxide consumed as a result of the behavior of phenolphthalein in ammoniacal solution.

### C. SOLUBLE IRON AND ALUMINA.

#### (a) METHOD OF SOLUTION.

All analysts of phosphate rock are familiar with the controversy over the best solvent for that part of the iron and aluminium which will be active and objectionable when the rock is converted into superphosphate. It can be said at the outset that the manufacturer of superphosphate is just as much in the dark as the chemist regarding the quantity of iron and aluminium in a given rock that is actually harmful, and that there is apparently no way to check theory against practice. In view of this fact, an attack with constant boiling hydrochloric acid (1:1) seems as satisfactory as any other. Nitric acid is not a good solvent for clayey matter and has the disadvantage of dissolving pyrite, which is not wanted, and leaving hematite, which is. Aqua regia usually causes practically complete solution and undoubtedly gives more "active iron and alumina", particularly iron, than is fair. At first thought it might seem that sulfuric acid would be most desirable; no doubt it would be if the rock did not contain fluorine. It is quite certain that hydrofluoric acid plays an important role during manufacture in dissolving the gangue matter in the moist hot mass after the rock is treated with sulfuric acid, but it should be remembered that the attack under these conditions will be far more effective than it will be in any laboratory test in which the hydrofluoric acid is diluted by a considerable volume of dilute sulfuric acid. The only reliable test of sulfuric acid would lie in a duplication of manufacturing conditions, and this is not practicable.

Glassware always contains more or less aluminium and sometimes zinc, and these are dissolved in part by hydrofluoric acid when phosphate rock is treated with acid in glass vessels. Both elements cause high results if "active" alumina is afterward determined by the phosphate method. The Fertilizer Division of the American Chemical Society ignored the

latter possibility and endeavored to overcome the former by specifying the use of a graduated flask containing less than 1 per cent of iron and aluminium oxides. This does not help much, as glassmakers' trade marks seldom appear on such flasks, and so there is no clue as to their composition. A much more effective remedy lies in the use of boric acid, which converts hydrofluoric acid into the less active fluoboric acid and reduces the attack very materially.

Other very important considerations in determining soluble iron and alumina are the temperature at which the solution is made and the duration of the attack. Entirely different quantities of iron and alumina, particularly alumina, will be obtained depending upon whether the solution is heated on the steam or water bath or actually boiled, or if the solution is boiled for 15 minutes as against one hour.

In view of the preceding considerations it is recommended that the solvent be dilute hydrochloric acid (1:1) containing boric acid, and that the solution be heated at approximately 110°C., which is the temperature usually reached when the boiling is done at ordinary pressures in a long-necked flask such as a measuring flask. In most of the work done at the Bureau of Standards the solution was boiled for one hour, as is usually specified. When the work was nearly finished it was decided that the long boiling period was of doubtful value and that *rigorous boiling* for 15 minutes might be substituted. The results obtained by such a procedure will be lower, it is true, but the probability is that they will be as fair as the results obtained in a longer digestion.

#### (b) SOLUBLE ALUMINA.

It is much more difficult to obtain concordant results for soluble alumina than for soluble iron. The difficulty lies in the method of solution rather than in the actual determination, as is shown in the following tests in which the actual quantities of alumina dissolved were accurately determined by tested methods. It will be seen that the results obtained by using a given method of solution check quite well, while those obtained after slightly differing treatments do not. For example, sample No. 56 showed 2.29 and 2.30 per cent of  $\text{Al}_2\text{O}_3$  when the rock was heated (actual temperature 80°C.) with dilute hydrochloric acid (1:1) in a platinum dish for one hour on the steam bath. When the experiment was repeated with the sole difference that the solutions were gently simmered (actual temperature 95°C.) the results were 2.55, 2.57, 2.60, and 2.68 per cent. The last value resulted because in this case the solution was heated a few minutes longer than the others. Solution of the sample by boiling in a long-neck quartz flask (actual temperature 110°C.) gave an average value of 2.76. Solution of the rock in a long-neck "Pyrex" flask with dilute hydrochloric acid (1:1) containing 1 gram of boric acid gave 2.51 and 2.52 per cent. In an attempt to duplicate factory conditions.



2.5 gram portions of rock were treated in covered platinum crucibles with 1.4 cc. of 53 deg. Baumé sulfuric acid that had been heated to 50°C. After standing overnight, the treated mass was washed out of the crucibles into beakers with dilute hydrochloric acid (1:1), and the temperature of the solution was quickly raised to boiling. In one case this boiling was continued for 1 minute, in another for 3 minutes, and in the third for 5 minutes. The results for alumina were 2.04, 2.07, and 2.14 per cent, respectively.

Final determination of aluminium as the phosphate ( $\text{AlPO}_4$ ) is recommended in spite of the admitted defects of the method. It has been found at the Bureau of Standards that the method is much improved if macerated paper is used and if the washing of the precipitate is controlled by the simple expedient of an occasional test with silver nitrate. When iron is precipitated with aluminium the method becomes less certain, and for this reason two methods are given; in the more accurate method iron and calcium are removed before aluminium is precipitated; and in the routine method iron is precipitated with the aluminium and the precipitation of calcium is held low by the use of a large excess of ammonium chloride.

#### (c) SOLUBLE IRON

No difficulty is experienced in obtaining concordant results for soluble iron, provided reasonably similar methods of solution are used and organic matter (which is practically always present in solution) is destroyed before the reduction of the iron. Erroneous results for iron will be caused by vanadium if stannous chloride is used as the reducing agent, and by vanadium, chromium, and titanium if reduction by zinc is employed. Although all of these elements have been found in phosphate rock, no serious difficulty arises as vanadium and chromium usually occur in very small quantities, and titanium does not interfere in the reduction generally used—that by stannous chloride. Of course a wide difference may result from the use of different acids, as for example in sample No. 56, which gives 3.30 per cent  $\text{Fe}_2\text{O}_3$  after an aqua regia attack and 2.55 per cent after one with dilute hydrochloric acid (1:1). In contrast, the addition of boric acid to the hydrochloric acid solvent makes no difference in the result as it does with alumina. In the experiments with a sulfuric acid attack, which are cited under soluble alumina, the respective determinations of soluble iron showed 2.45, 2.45, and 2.47 per cent.

The method of analysis recommended by the Fertilizer Division<sup>1</sup> (Zimmermann-Reinhardt) is satisfactory if organic matter is destroyed as specified. Equally good results can be obtained in a shorter time by destroying the organic matter by adding a slight excess of permanganate at the start and then boiling. Undoubtedly many analysts will now favor titration with dichromate and the use of diphenylamine as an internal indicator according to Knop<sup>2</sup>.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1915, 7: 446.

<sup>2</sup> *J. Am. Chem. Soc.*, 1924, 46: 263.

**(D) TOTAL FERRIC OXIDE, ALUMINA, AND LIME.****(a) TOTAL FERRIC OXIDE AND ALUMINA.**

Although these determinations are not made often, they are of general interest, especially as checks on the determinations of soluble iron and alumina. In this connection it was interesting to note that half of the cooperating analysts reported more soluble alumina in sample No. 56 than the total alumina content of the sample.

The methods of analysis follow along the lines laid down for soluble iron and alumina after the rock has been completely decomposed.

**(b) TOTAL LIME (AND MAGNESIA).**

This determination, while uncommon in the analysis of phosphate rock, is needed occasionally. Analyses can not be made by the usual procedures owing to the presence of phosphoric acid, and recourse is usually had to precipitation as sulfate in a sulfuric acid-alcohol solution followed by precipitation as oxalate. The authors have used direct precipitation of the oxalate in oxalic acid solution with good success. Precipitation in weak acid solution has been applied by others for materials containing little or no phosphorus, as for example by Meade for the analysis of cement<sup>1</sup>. Calcium can be thus easily separated from iron, aluminium, titanium, zirconium, and of course magnesium. Barium in moderate quantity is not precipitated, while strontium and manganese are incompletely thrown down. The solubility of calcium oxalate in dilute oxalic acid-ammonium oxalate solution is about the same as in the customary alkaline solution.

The determination of magnesium can follow that of calcium; all that is needed is the customary procedure after the addition of a few grams of citric acid to keep iron and aluminium in solution.

**III. METHODS OF ANALYSIS.****A. DETERMINATION OF MOISTURE.**

The following method is based on that described by the Fertilizer Division of the American Chemical Society<sup>2</sup> and is recommended for the determination of moisture and for the drying of the sample.

Place 5-10 grams of sample in a glass-stoppered weighing bottle of such a diameter that the depth of the sample does not exceed 0.5 cm. Dry for 1 hour at 105°C. in a well-ventilated oven. The passage of a current of dry air through the oven and the use of a similar bottle carried along with the sample as a counterpoise are desirable but not absolutely necessary. At the end of an hour, loosely stopper the bottle and cool in a desiccator. Momentarily raise the stopper, again stopper, and weigh. The loss of weight is called "moisture" though this probably does not represent the true moisture content on account of secondary changes.

<sup>1</sup> Portland Cement, 189, Chem. Pub. Co., 1906 e.l.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1915, 7: 446.

**B. DETERMINATION OF PHOSPHORIC ACID.***(a) Solution of the Sample.*

Transfer 2.5 grams of the 80-mesh rock to a 400 cc. beaker, add 30 cc. of hydrochloric acid (sp. gr. 1.19) and 10 cc. of nitric acid (sp. gr. 1.42), and boil to a sirupy consistency. Add 1 gram of boric acid and dissolve the residue, which should be nearly solid after cooling, in 5 cc. of concentrated nitric acid and 50 cc. of water. Heat to boiling, cool, filter into a 250 cc. graduated flask, wash the filter thoroughly with cold water, and dilute to the mark. This procedure eliminates practically all the silica, but it is necessary to filter soon after digestion in order to prevent re-solution of some of it.

*(b) Routine Gravimetric Method.**(Single Precipitation.)***SOLUTIONS REQUIRED.**

*Molybdate solution.*—Mix 100 grams of pure molybdic anhydride or 118 grams of 85 per cent molybdic acid with 400 cc. of water and add 80 cc. of ammonium hydroxide, sp. gr. 0.90. When solution is complete, filter and pour the solution slowly and with constant stirring into a mixture of 400 cc. of nitric acid, sp. gr. 1.42, and 600 cc. of water. Let settle for 24 hours and use the clear supernatant liquid.

*Ammonium nitrate solution (5 per cent).*—Dissolve 50 grams of ammonium nitrate in 950 cc. of water.

*Ammonium hydroxide—Ammonium citrate solution.*—Dissolve 25 grams of citric acid in 700 cc. of water and add 350 cc. of ammonium hydroxide, sp. gr. 0.90.

*Dilute ammonium hydroxide (1:20).*—Mix 50 cc. of ammonium hydroxide, sp. gr. 0.90, and 1000 cc. of water.

*Dilute hydrochloric acid (1:20).*—Mix 50 cc. of hydrochloric acid, sp. gr. 1.19, and 1000 cc. of water.

*Magnesia mixture.*—Dissolve 50 grams of  $MgCl_2 \cdot 6H_2O$  and 100 grams of  $NH_4Cl$  in 500 cc. of water. Add ammonium hydroxide in slight excess, let stand overnight, and filter if a precipitate appears. Make barely acid with hydrochloric acid and dilute to 1000 cc.

**PROCEDURE.**

Transfer a 50 cc. aliquot portion of the prepared solution (representing 0.5 gram of the rock) to a 250 or 300 cc. beaker or Erlenmeyer flask, add 15 cc. of nitric acid (sp. gr. 1.42), and nearly neutralize with ammonium hydroxide (sp. gr. 0.90). Add 125 cc. of ammonium molybdate solution and digest at 60°C. for 30 minutes with frequent stirring or shaking. Cool by immersing in tap water for 5 minutes, filter on a paper of close texture, keep as much of the precipitate as possible in the flask, and wash the precipitation vessel and the precipitate five times with a 5 per cent solution of ammonium nitrate. Set the filtrate and washings aside after thorough mixing and make sure that no further separation of phosphomolybdate occurs.

Dissolve the precipitate in the flask in 20 cc. of ammonium hydroxide-ammonium citrate solution. Pour this solution through the filter which contains the remainder of the yellow precipitate and catch the solution in a 250 cc. beaker. Wash the flask and paper several times with a dilute solution of ammonium hydroxide (1 : 20), next with a little hot water, and finally with hot dilute hydrochloric acid (1 : 20). The volume of the solution at this point should be between 100 and 150 cc. Neutralize the ammoniacal solution with hydrochloric acid, using litmus as indicator, and add 1 cc. of hydrochloric acid, sp. gr. 1.19, and 10 cc. of magnesia mixture per decigram of phosphorus. Now add ammonium hydroxide (sp. gr. 0.90) dropwise and with continuous stirring until the solu-

tion is ammoniacal and most of the phosphorus has been precipitated. Finally add 15 cc. more of ammonium hydroxide and allow the solution to stand for 4 hours or overnight at room temperature. The time of standing may be reduced to 2 hours if the solution is kept in a refrigerator or an ice-water bath. Transfer the precipitate to a 9 cm. Whatman No. 42 filter or its equivalent and wash with dilute ammonium hydroxide (1 : 20). Ignite the precipitate carefully and at as low a temperature as possible until the carbon has been destroyed. Finally ignite at approximately 1000°C. to constant weight, and calculate to  $P_2O_5$  by multiplying the weight of  $Mg_2P_2O_7$  by 0.6379.

(c) *Umpire Gravimetric Method.*

(Double Precipitation.)

In umpire analyses or analyses for purposes of standardization the molybdate precipitation should be allowed to stand 4 hours or preferably overnight, and the precipitation with magnesia mixture should be repeated as follows: after washing the first magnesium-ammonium phosphate precipitate several times with dilute ammonium hydroxide (1 : 20), dissolve it on the filter in 25 cc. of dilute hydrochloric acid (1 : 1) catching the solution in the original beaker containing the bulk of the precipitate. Wash the filter thoroughly with dilute hydrochloric acid (1 : 20) and dilute the solution to 100 cc. Add 2-3 cc. of magnesia mixture and then ammonium hydroxide slowly until a crystalline precipitate appears, and finally an excess of 3-5 per cent by volume. Allow to stand 4-6 hours or preferably overnight, filter on paper, wash, ignite in platinum, and weigh as previously indicated.

The magnesium-ammonium phosphate may be filtered on a platinum or porcelain Gooch crucible, fitted with a platinum or asbestos pad carefully made and ignited to constant weight.

The performance of a single precipitation method is well illustrated in Column 2 of Table 1, while that of the double precipitation method is shown in experiments 9-16 and 26-32 in Column 3 of the same table. Results obtained by the single precipitation method are usually from 0.1-0.3 per cent too high, while the results obtained by the double precipitation method should be accurate to  $\pm 0.05$  per cent.

(d) *Alkalimetric Method.*

(Routine.)

SOLUTIONS REQUIRED.

*Ammonium hydroxide* (sp. gr. 0.96, or approximately 6*N*).—Mix 400 cc. of ammonium hydroxide, sp. gr. 0.90, with 600 cc. of water. This solution should be checked with a hydrometer or by titration.

*Potassium nitrate solution* (1 per cent).—Dissolve 10 grams of potassium nitrate in 1000 cc. of water.

*Standard sodium hydroxide solution* (approximately 0.3 *N*).—Prepare a saturated solution of sodium hydroxide in a stoppered flask, let settle overnight, and pipet a clear 20 cc. portion. Dilute to 1000 cc. with recently boiled water, cool to room temperature, and standardize against the Bureau of Standards Standard Benzoic Acid No. 39b<sup>1</sup>. Dilute, if desirable; restandardize; and protect the solution from carbon dioxide. The phosphorus titre should be calculated from the ratio 23:1 and the performance of the solution and the method checked against phosphate rock of known  $P_2O_5$  content.

*Standard nitric acid solution* (approximately 0.3 *N*).—Mix 20 cc. of nitric acid (sp. gr. 1.42) and 1000 cc. of water, cool to room temperature, and standardize against the standard sodium hydroxide solution. Adjust the solution until it is exactly equivalent.

*Phenolphthalein solution* (1 per cent).—Dissolve 1 gram of pure phenolphthalein in 100 cc. of 85-95 per cent alcohol.

<sup>1</sup> *J. Am. Chem. Soc.*, 1912, 34: 1027; 1913, 35: 1309.

TABLE 1.

*Determination of phosphoric acid ( $P_2O_5$ ) in Bureau of Standards Sample No. 56.*

(All results refer to the sample dried at 105°C. Analyses 1-8 inclusive are by cooperating analysts and the remainder by Bureau of Standards analysts. Nos. 9-25 inclusive were obtained by Analyst 1 and the remainder by Analyst 2. All the results obtained at the Bureau are reported, and unless otherwise indicated the methods described in III B were used by the Bureau analysts.)

NO.	GRAVIMETRIC METHODS (WEIGHED AS $Mg_2P_2O_7$ )		ALKALIMETRIC METHODS PRECIPITATION AT—		
	Single Precipitation	Double Precipitation	60°-65°C	40°-50°C.	20°C
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	31.30				
2	31.66		31.13	31.14	
3	31.83 32.29 31.95 <sup>a</sup>	32.08	31.70 32.25 <sup>b</sup>	32.35 <sup>b</sup> 31.90 <sup>c</sup>	32.21 <sup>b</sup>
4					31.31 <sup>a</sup>
5	31.49	31.50			31.55 <sup>b</sup>
6	31.37 32.16 31.22	31.24	31.49 <sup>b</sup>	31.51 <sup>b</sup> 29.81 <sup>c</sup> 31.25 <sup>a</sup>	31.21 <sup>b</sup>
7			31.45 <sup>a</sup>	31.57 <sup>c</sup>	30.84 <sup>a</sup>
8				31.50	31.35
9	31.55	31.30	32.52 <sup>b</sup>	31.33	31.52 <sup>b</sup>
10	31.56	31.30	32.84 <sup>b</sup>	31.41	31.32 <sup>b</sup>
11	31.54	31.32	33.23 <sup>b</sup>	31.22	
12	31.59	31.29	32.87 <sup>b</sup>	31.16	
13	31.38	31.30		31.30	
14	31.46	31.28		31.21	
15	31.49 <sup>b</sup>	31.31		31.41	
16	31.56 <sup>b</sup>	31.35		31.43	
17	31.36 <sup>b</sup>			31.32	
18	31.30 <sup>b</sup>			31.32	
19	31.35 <sup>b</sup>			31.32	
20	31.34 <sup>b</sup>			31.16	
21	31.33 <sup>b</sup>			31.22	
22	31.41 <sup>b</sup>			32.46 <sup>d</sup>	
23	31.32 <sup>b</sup>			32.09 <sup>d</sup>	
24	31.46 <sup>b</sup>			32.02 <sup>b</sup>	
25				32.13 <sup>b</sup>	
26	31.61	31.35		31.24	
27	31.56	31.38		31.55	
28	31.44	31.34		31.22	
29	31.41	31.34		31.28	
30		31.30		31.75	
31		31.29		31.42	
32		31.32		31.76	
33				31.15	
34				31.16	
35				32.86 <sup>d</sup>	
36				31.68 <sup>d</sup>	

<sup>a</sup> Modified A. O. A. C. method.

<sup>b</sup> A. O. A. C. method.

<sup>c</sup> Bureau of Standards method.

<sup>d</sup> Washed with 5 per cent ammonium nitrate.

## PROCEDURE.

Transfer a 10 cc. or a 25 cc. portion of the prepared solution to a 300 cc. Erlenmeyer flask, add 25 cc. of nitric acid (sp. gr. 1.42), and dilute to 100 cc. Add to the solution 40 cc. of ammonium hydroxide (sp. gr. 0.96) and 50 cc. of ammonium molybdate solution, shake for 10 minutes, and let stand for 20 minutes. Filter on an 11 cm. paper of close texture, wash the flask and precipitate five times with 15–20 cc. portions of a 1 per cent solution of potassium nitrate; then wash the filter paper, containing most of the precipitate, five times with like portions of the same solution (about 200 cc. of wash solution in all). The paper should, of course, be carefully washed each time from the rim downwards and then allowed to drain completely before washing with the next portion of solution. The wash solution, as well as all others subsequently used, must be free from carbon dioxide. Return the paper and precipitate to the flask, add enough 0.3 N sodium hydroxide to decompose the precipitate and then approximately 2 cc. in excess. Dilute with 25 cc. of water, stopper, and shake until the precipitate is dissolved. Dilute to approximately 150 cc. with water, add 6 drops of a 1 per cent solution of phenolphthalein, and discharge the pink color with standard acid. Finish the titration by adding standard alkali until the reappearance of the pink color. Subtract the volume of acid used from the total volume of alkali and multiply the remainder by the titre of the alkali solution.

Experiments 9–21 in the fifth column of Table 1 indicate the performance of the method in the hands of an analyst who is familiar with it, while experiments 26–34 show its performance in the hands of an analyst who is not. Results obtained by this alkali-metric method should be accurate to  $\pm 0.3$  per cent of  $P_2O_5$  in general use, although an accuracy to  $\pm 0.15$  per cent and even better is not uncommon with experienced analysts.

## C. DETERMINATION OF SOLUBLE IRON AND ALUMINA.

## SOLUTIONS REQUIRED.

*Hydrochloric-boric acid mixture.*—Mix 500 cc. of hydrochloric acid (sp. gr. 1.19) and 500 cc. of water, add 20 grams of pure boric acid, and warm until the boric acid has dissolved.

*Dilute hydrochloric acid (1:5).*—Mix 100 cc. of hydrochloric acid, sp. gr. 1.19, and 500 cc. of water.

*Dilute hydrochloric acid (1:20).*—Mix 50 cc. of hydrochloric acid, sp. gr. 1.19, and 1000 cc. of water.

*Dilute sulfuric acid (1:5).*—Add 100 cc. of sulfuric acid, sp. gr. 1.84, slowly and with stirring to 500 cc. of water.

*Sodium hydroxide-carbonate solution.*—Dissolve 100 grams of sodium hydroxide and 10 grams of sodium carbonate in 1000 cc. of water.

*Ammonium acetate solution (25 per cent).*—Dissolve 250 grams of ammonium acetate in 750 cc. of water.

*Stannous chloride solution.*—Dissolve 50 grams of the crystallized salt in 100 cc. of hot concentrated hydrochloric acid and dilute to 1000 cc. or heat an excess of granulated tin (free from iron) in hot concentrated hydrochloric acid in a covered Erlenmeyer flask until the solution is saturated and dilute this solution with eight times its volume of water.

*Mercuric chloride solution.*—Prepare a cold saturated solution.

*Manganese solution.*—(a) Dissolve 200 grams of crystallized manganese sulfate in 1000 cc. of water. (b) Pour 400 cc. of concentrated sulfuric acid slowly and with constant stirring into 1300 cc. of water and then add 300 cc. of sirupy phosphoric acid, sp. gr. 1.71. Mix solutions (a) and (b).

*Standard permanganate solution.*—(Approximately 0.03 N). Dissolve 1 gram of potassium permanganate in distilled water, dilute to 1 liter, allow to stand for several weeks,

filter through asbestos which is free from chlorides and organic matter, and standardize against the Bureau of Standards Standard Sample of Sodium Oxalate No. 40b as follows:

In a 200 cc. beaker dissolve 0.1000 gram of sodium oxalate in 75–100 cc. of hot water (80°–90°C.) and add 10 cc. of (1:1) sulfuric acid. Titrate at once with 0.03 *N*  $\text{KMnO}_4$  solution, stirring the liquid vigorously and continuously. The permanganate must not be added more rapidly than 10–15 cc. per minute, and the last 0.5–1 cc. must be added dropwise, with particular care to allow each drop to be fully decolorized before the next is introduced. The excess of permanganate used to cause an end point color must be estimated by matching the color in another beaker containing the same bulk of acid and hot water. The temperature of the solution should not be below 60°C. by the time the end point is reached.

(a) *Solution of the Sample.*

Transfer 2.5 grams of the pulverized rock to a 250–300 cc. flask, add 50 cc. of hydrochloric acid-boric acid mixture, and *boil* the solution gently in such a manner as to avoid concentrating the solution to less than half its original volume. The temperature of the solution during the boiling should be approximately 110°C., and it is desirable to use a flask with a long narrow neck. If such a flask is not available, a funnel should be placed in the neck of the flask.

After boiling the sample of rock for one hour, dilute the solution to 100 cc. and filter into a graduated 250 cc. flask, washing the filter thoroughly with cold dilute hydrochloric acid (1:20). Cool the solution, dilute to the mark, and mix thoroughly.

(b) *Determination of Soluble Alumina.*

(Umpire Method.)

Transfer a 100 cc. portion (representing 1 gram of rock) of the prepared solution into a platinum dish and evaporate the solution nearly to dryness. Cool, add 15 cc. of dilute sulfuric acid (1:5), and evaporate until copious fumes of sulfuric acid appear. Cool, dilute with about 100 cc. of water, add 10 cc. of concentrated hydrochloric acid, and heat until sulfates are in solution. If silica is present, filter and wash the filter thoroughly with dilute hydrochloric acid (1:20). Add several drops of a saturated solution of potassium permanganate and boil 10 minutes to oxidize any ferrous iron and organic matter.

Nearly neutralize the solution, which should have a volume of about 200 cc., with sodium hydroxide and pour it slowly and with constant stirring into 160 cc. of a sodium hydroxide-carbonate solution. This may be done conveniently through a pipet. Transfer the solution and precipitate to a graduated 500 cc. flask, cool, and dilute to the mark. Filter through a dry 12.5 cm. No. 42 Whatman or similar paper, discard the first 10–20 cc., and then catch the filtrate in a 250 cc. flask, or graduate. In this procedure only a trace of calcium remains in solution.

Take exactly 250 cc. of the filtrate, representing 0.5 gram of the rock; acidify with hydrochloric acid; and add 10 cc. of the concentrated acid in excess, and then 1 gram of di-ammonium phosphate. Dilute to 500 cc. and add macerated paper. One 11 cm. No. 40 Whatman paper thoroughly shaken with about 100 cc. of distilled water or one-half of an S. and S. macerated paper tablet No. 292 yields about the right amount of macerated paper for one determination. The macerated paper prevents the gelatinous  $\text{AlPO}_4$  from coagulating in lumps from which sodium salts and excess phosphate can not be washed.

Add 2 drops of methyl orange indicator, make just alkaline with ammonia, and restore the pink color with several drops of dilute hydrochloric acid (1:5). Heat the solution to boiling and add 30 cc. of a 25 per cent solution of ammonium acetate. Continue the boiling for 5 minutes, filter on an 11 cm. No. 42 Whatman or similar paper, and wash with hot 5 per cent ammonium nitrate solution until 5 cc. of the washings gives an

almost indistinguishable opalescence with an acidified solution of silver nitrate. This test is better than a specified amount of washing because completeness of washing is dependent upon such factors as the size of the  $\text{AlPO}_4$  precipitate, quantity of macerated paper, and rapidity of filtration. In some cases as much as 400 cc. of a 5 per cent solution of ammonium nitrate is required, while in others as little as 150 cc. suffices.

Dry the paper and precipitate by gentle heating in an open platinum or porcelain crucible, burn off the carbon at a low temperature, and finally cover and ignite at about  $1000^\circ\text{C}$ . to constant weight. Weigh as  $\text{AlPO}_4$  and multiply by 0.418 to get the weight of  $\text{Al}_2\text{O}_3$ .

Unless the reagents are known to be free from aluminium, a "blank" run should be carried through all steps of the method. The results obtained by different analysts should check to  $\pm 0.2$  per cent of  $\text{Al}_2\text{O}_3$ .

The accuracy of the method as applied to synthetic solutions is shown in Table 2. There is no way of checking its accuracy when applied to the analysis of phosphate rock, for here everything depends on the solution treatment. It will be noted, however, that in the five determinations (Nos. 13, 14, 15, 25, and 26, Column 3, Table 3) made by the Bureau of Standards analysts the maximum variation was 0.3 per cent of  $\text{Al}_2\text{O}_3$ , and the average deviation from the average was 0.08 per cent.

(c) *Determination of Soluble Iron.*  
(Umpire Method.)

Transfer a 100 cc. portion (representing 1 gram of rock) of the prepared solution to a 250 cc. beaker. If only a small quantity of organic matter is present, as is usually the case, add 1-2 cc. of a saturated solution of potassium permanganate and then boil to

TABLE 2.

*Tests of method C(b), which is recommended for the accurate determination of soluble alumina.*

(All solutions were acidified, treated with 50 cc. of 1:1 HCl, boiled for 1 hour in a Pyrex flask, then heated with sulfuric acid in platinum until fumes appeared, and finished exactly as in the method.)

EXPERIMENT	SUBSTANCES ADDED	$\text{Al}_2\text{O}_3$	
		Added	Found
1	None	gram 0.0154	gram 0.0156
2	0.5 gram $\text{H}_3\text{BO}_3$	0.0154	0.0154
3	0.2 gram NaF	0.0154	0.0171 *
4	{ 0.2 gram NaF 0.5 gram $\text{H}_3\text{BO}_3$ }	0.0154	0.0158
5	{ 8 grams NaOH <sup>b</sup> 0.5 gram $\text{Na}_2\text{CO}_3$ }	0.0154	0.0147
6		0.0385	0.0385
7	{ 0.44 gram CaO 0.034 gram $\text{Fe}_2\text{O}_3$ 0.30 gram $\text{P}_2\text{O}_5$ }	0.0154	0.0157
8	{ 0.2 gram NaF 0.2 gram $\text{H}_3\text{BO}_3$ }	0.0385	0.0389

\* High result probably caused by hydrofluoric acid attack on glass.

<sup>b</sup> To determine the effect of excessive amounts of alkali salts.



expel chlorine. In the rare event of much organic matter, add a few crystals of potassium chlorate at intervals as the solution is evaporated to dryness and redissolve the residue in 30 cc. of dilute hydrochloric acid (1:5).

Heat the solution to boiling and reduce with a small excess of stannous chloride, added dropwise while agitating the solution. Wash the sides of the beaker with distilled water and cool rapidly. Add 10 cc. of mercuric chloride solution and stir vigorously for one minute. Pour the mixture into a large porcelain casserole containing 20 cc. of the manganese solution in about 500 cc. of water that has been faintly tinted with permanganate solution. Titrate with 0.03 *N* permanganate solution to the original tint, and correct the result by the volume of permanganate required for a titration of the reagents alone.

Calculate the percentage of  $\text{Fe}_2\text{O}_3$  that is indicated.

The results obtained by the above method should be accurate to  $\pm 0.05$  per cent. The performance of the method is indicated in experiments Nos. 12-15 in Column 2 in Table 3.

(d) *Determination of Soluble Iron and Alumina.*

(Routine Method.)

The following modification of the method given by the Fertilizer Division is rapid and should be acceptable for routine purposes. It will be noted (1) that a large excess of ammonium chloride is used in order to reduce the precipitation of  $\text{Ca}_3(\text{PO}_4)_2$  to a minimum; (2) that macerated paper, which aids filtration and washing, is added instead of a solution of iron which complicates this procedure; and (3) the washing of the precipitate is definite and not empirical.

(1) SOLUBLE IRON AND ALUMINA.

Prepare the solution exactly as described above and transfer a 50 cc. aliquot, representing 0.5 gram of rock, into a platinum dish. Evaporate nearly to dryness, cool, add 15 cc. of dilute sulfuric acid (1:5), and evaporate till nearly all the sulfuric acid has been driven off. Cool, dilute with 75 cc. of water, add 10 cc. of concentrated hydrochloric acid, and heat until the sulfates are in solution. Filter into an 800 cc. beaker and wash the paper thoroughly with dilute hydrochloric acid (1:20).

Add 100 cc. of a saturated solution of ammonium chloride, 3-4 cc. of a 10 per cent solution of di-ammonium phosphate (freshly prepared), 2 drops of methyl orange indicator, and macerated paper. One tablet of S. and S. macerated paper or one 11 cm. No. 40 Whatman paper shaken to a pulp furnishes about the proper amount of paper pulp for one determination. Dilute to 500 cc., render the solution ammoniacal, and just restore the pink color with dilute hydrochloric acid (1:5). Heat to boiling and add 30 cc. of a 25 per cent solution of ammonium acetate. Boil for 5 minutes and filter immediately on a 12.5 cm. ashless filter. Wash with a hot 5 per cent solution of ammonium nitrate until 5 cc. of the washings shows only a barely perceptible opalescence with acidified silver nitrate; 350-450 cc. of wash solution is ordinarily required.

Ignite the precipitate as described in the previous method and finally weigh as combined  $\text{AlPO}_4 + \text{FePO}_4$ .

(2) SOLUBLE IRON.

Determine iron as previously described in a 100 cc. aliquot of the same solution as was used in (1) above and calculate the percentage of  $\text{Fe}_2\text{O}_3$ .

(3) SOLUBLE ALUMINA.

Calculate the  $\text{FePO}_4$  equivalent from the weight of  $\text{Fe}_2\text{O}_3$  found in (2), subtract from the combined weight of  $\text{AlPO}_4 + \text{FePO}_4$  obtained in (1), and calculate  $\text{Al}_2\text{O}_3$  in the remainder by multiplying by 0.418.

## (4) EMPIRICAL CALCULATION OF SOLUBLE IRON AND ALUMINA.

Some analysts report combined soluble iron and alumina as obtained by dividing the percentage of the combined phosphates by 2. Such a practice is not objectionable in routine analyses, considering the nature of the determination, provided the percentages are small and *all* analysts agree to use this procedure. If universal agreement to the procedure can not be obtained, it should be discarded.

The phosphate precipitates obtained in (1) usually contain from 0.001–0.002 gram of tri-calcium phosphate, which is counted as soluble alumina if calculation is made as in (3) or as soluble iron and alumina if calculation is made as in (4). In addition, the ferric phosphate practically always contains an excess of  $P_2O_5$ , which affects the results in the same way.

Results for soluble alumina obtained by calculation as in (3) should check to  $\approx 0.2$  per cent and will show more alumina than was present in the solution. For example, aliquots of the same solution showed 2.82 and 2.89 per cent of alumina by this method, as against a true content of 2.58 per cent as determined by the umpire method.

Results for soluble iron should be as accurate as in the umpire method.

Results obtained as in (4) will ordinarily be higher than the sum of the results obtained by the umpire methods. For example, 5.83 per cent was thus indicated by method (4) instead of the true value 5.07 per cent in a solution of the rock prepared as in the umpire method C (a) (one hour boiling with 1:1 HCl plus boric acid). When the boiling period was shortened to 15 minutes, method (4) showed 5.08 per cent. These results should be compared with an average value of 5.31 per cent which was obtained by the umpire methods after omitting boric acid in the solution treatment.

In Table 3 are shown the results obtained in the analysis of sample No. 56 by different methods. Analyses 1–8 were obtained by the cooperating analysts, and it is not certain that their results labeled (c) were obtained by strict adherence to the Bureau of Standards umpire method. Analyses 9–22 were made by Analyst No. 1, and the others were made by Analyst No. 2 at the Bureau of Standards. For purposes of comparison it should be remembered that the sample contains 3.30 per cent of total  $Fe_2O_3$  and 3.07 per cent of total  $Al_2O_3$ .

**D. DETERMINATION OF TOTAL FERRIC OXIDE, ALUMINA, LIME, AND MAGNESIA.***(a) Preparation of the Solution.*

Transfer 2.5 grams of the rock to a platinum dish (on account of the fluorides present) and digest for 15–30 minutes on the steam bath, with 50 cc. of dilute hydrochloric acid (1:1). Remove any insoluble matter by filtration, ignite in platinum, fuse with a small quantity of sodium carbonate, take up the melt in dilute hydrochloric acid, and add the solution to the filtrate. Remove the silica by two evaporations in platinum with hydrochloric acid and intervening filtration. If considerable quantities of fluorides are present, dust a little powdered silica (free from iron, aluminium, calcium, and magnesium) into the solution from time to time during the evaporation with hydrochloric acid. Ignite the

TABLE 3.

*Determination of soluble iron and alumina in Bureau of Standards Sample No. 56.*

	SOLUBLE IRON (Fe <sub>2</sub> O <sub>3</sub> )	SOLUBLE ALUMINA	SOLUBLE IRON AND ALUMINA (Fe <sub>2</sub> O <sub>3</sub> + Al <sub>2</sub> O <sub>3</sub> )	METHODS USED FOR IRON AND ALUMINA
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	2.60 <sup>a</sup>	3.39 <sup>b</sup>	5.99	<sup>a</sup> Method of Fertilizer Division <i>J. Ind. Eng. Chem.</i> , 1915, 7: 447.
2	1.99 <sup>a</sup>	3.18 <sup>a</sup>	5.17	
3		2.95 <sup>c</sup>	5.61 <sup>d</sup>	<sup>b</sup> Separated alumina with KOH then precipitated with ammonia and ammonium phosphate.
4	2.58 <sup>a</sup>	2.65 <sup>b</sup>	6.35 <sup>a</sup>	<sup>c</sup> B. S. umpire method.
5	2.83 <sup>a</sup>	3.11 <sup>c</sup>	5.23	<sup>d</sup> Treated rock with HCl, separated calcium with alcohol, and precipitated as phosphate.
6	2.65 <sup>a</sup>	3.30 <sup>a</sup>	5.94	
7		3.02 <sup>c</sup>	5.95	
8	2.89 <sup>a</sup>	3.05 <sup>c</sup>	5.94	<sup>e</sup> Reduction with zinc and titration with KMnO <sub>4</sub> .
			6.45 <sup>a</sup>	<sup>f</sup> Method of the Fertilizer Division with addition of paper pulp instead of iron.
			5.50 <sup>f</sup>	
			6.50 <sup>a</sup>	<sup>g</sup> Dissolved in HNO <sub>3</sub> and HCl and then applied method of the Fertilizer Division.
			4.00 <sup>h</sup>	
			4.60 <sup>b</sup>	<sup>h</sup> Glaser alcohol method.
			5.80 <sup>b</sup>	<sup>i</sup> Rock simmered in platinum for 1 hour with HCl (1:1) and then B. S. umpire method applied without the addition of boric acid.
			4.20 <sup>b</sup>	<sup>j</sup> B. S. umpire method.
9	2.55 <sup>a</sup>	3.08 <sup>a</sup>	5.63	
10	2.56 <sup>a</sup>	3.09 <sup>a</sup>	5.65	<sup>k</sup> Rock boiled in quartz flask for 1 hour with HCl (1:1) without addition of boric acid and then B. S. umpire method applied.
11	2.54 <sup>a</sup>	2.57 <sup>i</sup>	5.11	
12	2.56 <sup>j</sup>	2.68 <sup>i</sup>	5.24	<sup>l</sup> B. S. routine method.
13	2.55 <sup>j</sup>	2.71 <sup>c</sup>	5.26	
14	2.54 <sup>j</sup>	2.51 <sup>c</sup>	5.05	<sup>m</sup> B. S. routine method but boiled rock with HCl (1:1) and H <sub>2</sub> BO <sub>3</sub> for only 15 minutes.
15	2.55 <sup>j</sup>	2.52 <sup>c</sup>	5.07	<sup>n</sup> Method of Fertilizer Division but used quartz flask for solution of rock.
16		2.84 <sup>k</sup>		
17		2.78 <sup>k</sup>		
18		2.82 <sup>k</sup>		
19	2.55	2.82 <sup>l</sup>	5.37 <sup>l</sup>	
20	2.55	2.89 <sup>l</sup>	5.44 <sup>l</sup>	
21	2.55	2.25 <sup>m</sup>	4.80 <sup>m</sup>	
22	2.55	2.21 <sup>m</sup>	4.76 <sup>m</sup>	
23	2.54 <sup>a</sup>	3.26 <sup>a</sup>	5.80	
24	2.54 <sup>a</sup>	3.30 <sup>a</sup>	5.84	
25	2.51 <sup>a</sup>	2.45 <sup>c</sup>	4.96	
26	2.56 <sup>a</sup>	2.41 <sup>c</sup>	4.97	
27		2.73 <sup>k</sup>		
28		2.65 <sup>k</sup>		
29		3.10 <sup>n</sup>		
30		2.85 <sup>f</sup>		

silica and treat it with sulfuric and hydrofluoric acids. If any residue remains, fuse it with a little sodium carbonate, dissolve the melt in a small quantity of dilute hydrochloric acid, and add the solution to the main filtrate. Dilute the solution to exactly 250 cc., mix thoroughly, and pipet 50 cc. aliquot portions, representing 0.5 gram of sample, into beakers for determinations of total ferric oxide and of lime, and into platinum dishes for the determination of total alumina.

(b) *Total Ferric Oxide.*

Total ferric oxide is conveniently determined in one aliquot portion by Method C (c) which is given for "Soluble Iron" after provision has been made for any platinum introduced through the solution treatment given in (a). Platinum interferes in permanganate titrations that are made after reductions with stannous chloride and causes high results.

If platinum is removed by precipitation with hydrogen sulfide, another difficulty arises through the formation of polythionic compounds which also react with permanganate and cause high values. The latter are removed by boiling to expel hydrogen sulfide and then continuing the boiling as 2 cc. of a 5 per cent solution of permanganate is added.

(c) *Total Alumina.*

Total alumina is satisfactorily determined in another aliquot portion of the solution prepared in (a) by Method C (b) for "Soluble Alumina", provided a double precipitation of the aluminium as phosphate is made. In the first precipitation macerated paper can be omitted, and the washing of the precipitate need be only very slight. The error introduced by the volume of the precipitate in the sodium hydroxide precipitation is negligible. To confirm the result for alumina, the aluminium phosphate can be dissolved and its  $P_2O_5$  content carefully determined and then subtracted from the weight of  $AlPO_4$ .

(d) *Total Lime.*

To an aliquot portion of the solution prepared in (a) add sufficient hydrochloric acid to make a total of 10 cc. of concentrated hydrochloric acid in the solution. Heat to about  $50^{\circ}C.$ , add several drops of methyl orange, neutralize with ammonium hydroxide, and add 1 cc. of dilute ammonium hydroxide (sp. gr. 0.96) in excess. Just acidify the solution with oxalic acid (10 per cent solution) and then add 10 cc. in excess. Heat the solution to boiling, stir vigorously, and allow to boil 1-2 minutes. It is necessary to boil and to stir vigorously after the addition of oxalic acid in order to free the calcium oxalate precipitate from iron, aluminium, etc., which may at first be thrown down in part.

Add 50 cc. of a saturated solution of ammonium oxalate (about a 4 per cent solution), dilute to 200 cc. with hot water, boil for one minute, and place on the steam bath for one hour. Cool to room temperature, filter, and wash several times with cold ammonium oxalate-oxalic acid wash solution (2 grams  $(NH_4)_2C_2O_4$  and 1 gram  $H_2C_2O_4 \cdot 2H_2O$  per 1000 cc. of water). Reserve the filtrate for the determination of magnesia.

Ignite the precipitate in platinum, dissolve in 40 cc. of dilute hydrochloric acid (1:4), and filter if any silica separates. Dilute to 200-250 cc., add 0.01 gram of  $FeCl_3$ , make slightly ammoniacal, and add 10 cc. of bromine water. Digest at a temperature a little below the boiling point for 15 minutes and then add 5 cc. more of bromine water. Allow to stand on the steam bath for  $\frac{1}{2}$ -1 hour, filter, and wash thoroughly with hot ammonium-ammonium chloride wash solution (10 cc. of strong ammonium hydroxide and 10 grams of ammonium chloride per liter). Acidify the filtrate with hydrochloric acid, add 25 cc. of a saturated solution of ammonium oxalate, heat nearly to boiling, and then make ammoniacal, adding about 1 cc. of strong ammonium hydroxide in excess. Boil for 1-2 minutes, allow to stand on the steam bath for 1 hour, cool to room temperature, filter, and wash with a cold 0.4 per cent solution ammonium oxalate.

Ignite the precipitate, and weigh as  $CaO$  in a covered crucible. Repeat the ignition and weighing until constant weight is obtained.

The above method gives results that are comparable with those obtained by the ordinary oxalate method in rock analysis. Because of the solubility of calcium oxalate a little is unprecipitated in both methods, but the total amount need not exceed more than 0.5 mg. (0.1 per cent  $CaO$  in a 0.5 gram sample) in double precipitations. In the accurate analysis of minerals this is subsequently recovered by treatment of the weighed magnesium pyrophosphate; in the analysis of phosphate rock this refinement is not necessary.

TABLE 4.  
Performance of Method D (d) with known mixtures.

SUBSTANCES ADDED	LIME	
	Present	Found
gram 0.06 MgO	gram 0.0588	gram 0.0585 0.0584
0.02 Fe 0.008 Al 0.001 Zr 0.001 Ti 0.004 Mg 0.1 P <sub>2</sub> O <sub>5</sub>	0.0588    0.0015	0.0590 <sup>a</sup> 0.0588 <sup>a</sup>   0.0011 0.0011
0.04 Fe 0.008 Al 0.001 Zr 0.001 Ti 0.004 Mg 0.15 P <sub>2</sub> O <sub>5</sub>	0.0588	0.0588 0.0590
0.04 Fe 0.008 Al 0.001 Zr 0.001 Ti 0.007 Mg 0.005 Mn 0.15 P <sub>2</sub> O <sub>5</sub>	0.1470	0.1468 <sup>b</sup> 0.1467 <sup>b</sup>
2.00 Na <sub>2</sub> CO <sub>3</sub>	0.0588	0.0589
0.005 Ba	0.0588	0.0591
0.005 Ba 0.03 P <sub>2</sub> O <sub>5</sub>	0.0294	0.0293
0.005 Sr	0.0588	0.0625
0.005 Sr 0.03 P <sub>2</sub> O <sub>5</sub>	0.0294	0.0324
B. S. Limestone Sample No. 1 plus 0.30 gram P <sub>2</sub> O <sub>5</sub>	37.65 <sup>c</sup>	37.66 37.75 37.70
British Chemical Standard Basic Slag "A"	44.73 <sup>c</sup>	44.56

<sup>a</sup> Contained none of the added substances.

<sup>b</sup> Contained 0.0001 gram Mn.

<sup>c</sup> General average on certificate.

The treatment with iron, ammonium hydroxide, and bromine serves quite satisfactorily for the removal of manganese and residual phosphoric acid, and numerous tests have shown that usually less than 0.2 mg. of CaO is carried down with the precipitate. Table 4 shows the performance of the method as applied to mixtures of known lime content.

The performances of methods D(b), D(c), and D(d) are shown in Table 5, which lists the results obtained by their use at the Bureau of Standards for the analysis of the Bureau's Phosphate Rock Sample No. 56.

In conclusion the authors wish to express their indebtedness to W. F. Hillebrand for helpful advice and to H. B. Knowles for aid in the analyses.

TABLE 5.

*Determination of total  $Fe_2O_3$ ,  $Al_2O_3$ , and  $CaO$  in Sample No. 56.*

( $Fe_2O_3$  and  $CaO$  were determined as in D(b) and D(d);  $Al_2O_3$  was determined as shown below )

NO	TOTAL			METHODS USED FOR $Al_2O_3$
	$Fe_2O_3$	$Al_2O_3$	$CaO$	
1	3.26	3.06 <sup>a</sup>	44.77	<sup>a</sup> Iron and aluminium precipitated as phosphates, $P_2O_5$ removed with molybdate, iron and aluminium precipitated with ammonia, aluminium precipitated with phenylhydrazine. $Al_2O_3$ corrected for titanium. In a mixture approximating the composition of the rock this method gave 0.0613 gram $Al_2O_3$ as against 0.0616 gram added.
2	3.30	3.08 <sup>a</sup>	44.89	
3	3.31	3.03 <sup>b</sup>		
4	3.33	3.11 <sup>b</sup> 3.06 <sup>c</sup>		<sup>b</sup> Iron and aluminium twice precipitated as phosphates, iron removed by double precipitation with $NaOH$ , and aluminium determined by double precipitation as phosphate in combined filtrates.
5	3.31	3.12 <sup>c</sup> 3.03 <sup>d</sup>		<sup>c</sup> Result obtained by difference after determining $P_2O_5$ in the $AlPO_4$ obtained in (b). $AlPO_4$ was dissolved in $HCl$ , and $P_2O_5$ twice precipitated by magnesia mixture in the presence of citric acid.
6	3.28	3.04 <sup>d</sup> 3.02 <sup>d</sup>		<sup>d</sup> Result obtained after separation of $P_2O_5$ as in (c). Citric acid destroyed with $HNO_3-H_2SO_4$ . $Al_2O_3$ precipitated with phenylhydrazine and corrected for $TiO_2$ .
7	3.25	3.10 <sup>e</sup> 3.04 <sup>e</sup>		<sup>e</sup> Umpire method D(c).

## SUMMARY

1. Methods for the determination of moisture, phosphoric acid, "soluble iron and alumina", and calcium are given, together with results obtained in the analysis of the Bureau of Standards standard sample of phosphate rock by the use of these and other methods.

2. The use of boric acid is desirable in preparing solutions for the analysis of phosphate rock, for this lessens the hydrofluoric acid attack on glassware and prevents the interference of this acid in determinations of phosphorus.

3. Results obtained by solution of the ammonium-phosphomolybdate precipitate and a single precipitation with magnesia mixture can not be correct except through compensating errors, for the precipitate always contains molybdenum and is rarely of ideal composition. If magnesia mixture is added too slowly to neutral or ammoniacal solutions of phosphate, the results are usually low. For accurate analyses, solution of the magnesium ammonium phosphate precipitate is recommended, followed by the addition of 2-3 cc. of magnesia mixture and reprecipitation by ammonia.

4. In the alkalimetric method for phosphorus, the solution should not be heated after the addition of the molybdate reagent. The 23 : 1 ratio can not be used in calculating the phosphorus titre of the sodium hydroxide solution unless the method is carefully worked out and followed to the letter.

5. It is not difficult to get concordant results for "soluble iron". The determination of "soluble alumina", on the other hand, requires rigid attention to a definitely defined method of solution and careful analysis if comparable results are to be obtained.

### A STUDY OF THE ROTATIONS PRODUCED BY LEMON OIL AND SWEET ORANGE OIL, RESPECTIVELY, WHEN IN ALCOHOLIC SOLUTION.

By WYATT W. RANDALL (Bureau of Chemistry, State Department of Health, Baltimore, Md.).

According to the official methods<sup>1</sup> the percentage of oil by volume in a lemon extract may be found by dividing the rotation in terms of Ventzke degrees, determined at 20°C. in a 200 mm. tube, by the figure 3.2. Similarly, the percentage of oil by volume in an orange extract may be found by dividing the rotation, determined under the same conditions, by 5.2. Since a rotation of 1° of arc is equivalent to one of 2.8854°V., the percentage of oil by volume would be found, in the case of these two extracts, by dividing the rotations in degrees of arc by 1.11 and by 1.80, respectively.

To obtain the percentage of oil by volume, according to Leach<sup>2</sup>, the reading in degrees V. should be divided by 3.4, in the case of lemon extract, and by 5.3, in the case of orange extract. This would mean dividing the rotations in degrees of arc by 1.18 and by 1.84, respectively—or in minutes of arc by 70.8 and by 110.4, respectively. Yet Leach also states that the divisor for readings in minutes of arc should be 62.5 in the case of lemon extract. Woodman<sup>3</sup> states that the percentage of oil by volume in lemon extract will be found by dividing the rotation in degrees V. (under the conditions given above) by 3.2, or that in minutes of arc by 68. Here again there is a slight discrepancy: if 3.2 is correct for degrees V., then the divisor for minutes of arc should be 66.6. Thurston<sup>4</sup> gives the divisors 3.2 and 5.2, and Winton<sup>5</sup> and Leffmann and Beam<sup>6</sup> give the figure 3.2 as the divisor for lemon extract.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 200.

<sup>2</sup> *Food Inspection and Analysis*, 4th ed., 1920, pp. 930, 942.

<sup>3</sup> *Food Analysis*, 1915, p. 400.

<sup>4</sup> *Pharmaceutical and Food Analysis*, 1922, pp. 316, 350.

<sup>5</sup> *Food Analysis*, 1917, p. 198.

<sup>6</sup> *Select Methods in Food Analysis*, 1905, p. 325.

In all these books the figures given for lemon extract seem to have been based upon the results obtained by A. S. Mitchell<sup>1</sup>. In his paper Mitchell says: "From these figures it will be seen that oil of lemon will produce a dextrorotation of about  $3.4^{\circ}$  [V.] for each per cent of oil in alcoholic solution under the above conditions". Again, he speaks of "dividing the result in degrees by 3.4", in spite of the fact that the actual results given in the article call for the use of a divisor 3.27 rather than 3.4. In Bulletin 65<sup>2</sup>, 3.2 is the divisor recommended by Mitchell; nevertheless the editor of Leach has not adopted this revision of the figure.

In view of this lack of uniformity among the authorities, it seemed worth while to make an investigation of the rotations characteristic of lemon oil, of sweet orange oil, and of extracts prepared from these. Experiments were accordingly made upon the following oils:

Lemon oil I (Sanderson's), imported by Dodge and Olcott Co.

Lemon oil II, U. S. P., "F. B. Handpressed": Fritzsche Bros., Inc.

Sweet orange oil III (Sanderson's), imported by Dodge and Olcott Co.

Sweet orange oil IV, U. S. P., "F. B. Handpressed": Fritzsche Bros., Inc.

Sweet orange oil V, U. S. P., "Coldpressed California": Fritzsche Bros., Inc.

NOTE.—After the work here described had been completed and this article written in practically its present form, the author's attention was directed to the Report on Flavoring Extracts, by A. E. Paul<sup>3</sup>, which in some way he had overlooked in his search of the literature. This report contains a series of results secured by C. B. Gnadinger with samples of the oils of lemon and sweet orange and of extracts prepared from them. Had the writer of this paper been aware of Paul's report, he would probably not have undertaken the work here described; as it is, he has thought it might be well to set down the results of his measurements.

#### APPARATUS USED.

*Polarimeter.*—This instrument is of Pellin's make, with scales for reading both in angular degrees and in Ventzke degrees; the manufacturer guarantees accuracy to  $2'$  of arc. The bed is long enough to accommodate a 500 mm. tube. A burner of the Meker type gives a powerful sodium light; even with the polarizer adjusted to extreme sensitivity, readings were satisfactorily made with a column of 500 mm. of 5 per cent extract with one exception—with Sample V a 200 mm. tube was used. The oils themselves, with the same exception, were examined in 200 mm. tubes; owing to its strong color, it was necessary to use a 100 mm. tube for Sample V.

*100 cc. flask.*—This was calibrated by filling it to the mark with recently boiled water at exactly  $30^{\circ}\text{C}$ . The water contents weighed 99.4242 grams in air; at  $20^{\circ}$  the flask would contain 99.936 cc.

<sup>1</sup> *J. Am. Chem. Soc.*, 1899, 21: 1132.

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull. 65.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1921, 4: 468.



*Pipet.*—A pipet "to contain", of approximately 5 cc. capacity and of the shape shown in Fig. 1, was used; the wire by which the pipet was hung on the balance hook is not shown, however. The mark is at M, on the capillary part of the suction tube. Liquid is drawn in until it reaches nearly to A; the pipet is then hung in a constant-temperature bath, the liquid now occupying the space between A and B while this portion of

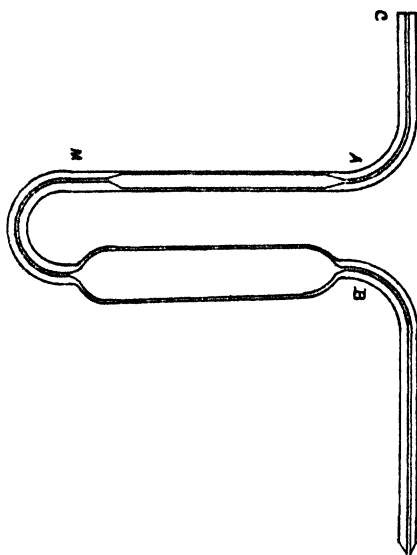


FIG. 2—PYCNOMETER USED.

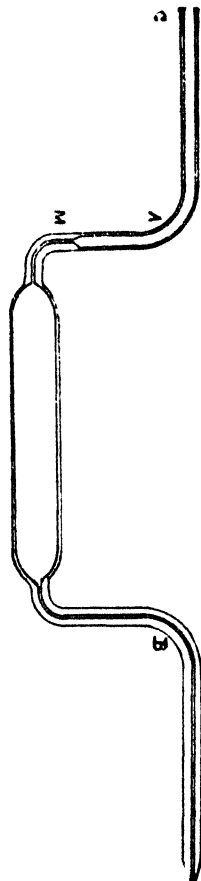


FIG. 1—PIPET USED.

the pipet is immersed in the water of the bath. When proper temperature conditions have been secured, a short piece of rubber tubing, closed at one end by a glass plug, is carefully pushed on the end C until the liquid begins to issue from the tip and the meniscus reaches M; the tip is then wiped off with a bit of filter paper and the rubber cap withdrawn from C. The liquid now adjusts itself in the two arms, and, if desired, weighing may be made after the pipet and its contents have come to room temperature. The contents of the pipet can then be drained into the volumetric flask, rinsing being accomplished with alcohol.

As the work described in this paper was carried out while the air temperature ranged from about 75° to 94°F., a pipet or a pycnometer that could be filled at 15°C., and yet would not overflow when its contents had become warmed to 33°C., was considered desirable.

Three determinations of the capacity of this pipet were made, two at 20° and one at 29.5°C. The first two gave 5.101 cc. and 5.096 cc., respectively; the third, corrected for temperature, gave 5.099 cc.: average, 5.099 cc. This pipet was used for preparing 5 per cent extracts, but since its shape made it an awkward instrument to weigh another form was adopted for the pycnometer and for the 1 cc. pipet.

*Pycnometer*—The 10 cc. pycnometer was of the form shown in Fig. 2. Like the pipet, it could be filled by suction until the liquid reached nearly to A. The apparatus was then hung in the constant-temperature bath as long as was necessary; the rubber cap was pushed upon C until the meniscus had been brought to M; the liquid forced from the tip was removed; the cap was withdrawn; and the pycnometer and its contents were allowed to come to room temperature, wiped off, and weighed. In this way any overflow due to expansion of the liquid as the temperature rose is avoided, as well as error due to deposited atmospheric moisture.

An approximately 1 cc. pipet, similar in shape to the pycnometer, was also made and used in the preparation of 1 per cent extracts. Calibration with water showed its capacity to be 1.038 cc.

## EXTRACTS

With the aid of the 5 cc. pipet a 5 per cent extract was made from each of the oils listed. The pipet was filled with the oil at 20°C., and the oil was drained into the 100 cc. flask. While the pipet was in an upright position alcohol was repeatedly run into the end C until all the oil had been rinsed from it. The flask was then placed in the bath, and ordinary alcohol was added in successive portions until the meniscus stood at the mark when the flask contents were at 20°C. Finally enough alcohol was added to yield a total volume of 102 cc.—20 times the capacity of the pipet. In a similar way some 5 per cent extracts were prepared with absolute alcohol and, with the aid of the 1 cc. pipet, several of 1 per cent concentration.

## PROPERTIES OF THE SEVERAL OILS.

In the case of each of the five oils mentioned previously the determinations of the angle of rotation were made at air temperature; care was taken, however, by means of a thermometer in the polarimeter trough, to note any temperature variation. Tube and thermometer were wrapped in the same cover. The observed rotations were calculated to the values at 20°C. on the assumption that, in a 100 mm. tube, for each centigrade

degree above 20° the rotation decreases 8.2' of arc in the case of lemon oil, and 13.2' of arc in the case of sweet orange oil<sup>1</sup>. A 200 mm. tube was used in each case except that of the California orange oil, as noted previously. In each case the result given in Table 1 is the average of at least ten readings, reduced to the 100 mm. basis.

TABLE 1.  
*Oils: Rotations in degrees of arc at 20°C. (100 mm. tube).*

OIL	NUMBER OF READINGS	AVERAGE
		<i>degrees</i>
Lemon oil I. ....	18	61.566
Lemon oil II. ....	10	61.884
Sweet orange oil III. ....	10	96.800
Sweet orange oil IV. ....	10	98.115
Sweet orange oil V. ....	10	97.975

*Oils: Specific gravities at 15°/15° (in air).*

Lemon oil I. ....	0.8582
Sweet orange oil III. ....	0.8504

#### PROPERTIES OF THE SEVERAL EXTRACTS.

The rotations produced by the oils in their alcoholic solutions were determined at air temperature in the 500 mm. tube, with the exception of the California orange oil extract, which, owing to its strong color, was examined in a 200 mm. tube. The results were calculated to the 200 mm. column basis, use being made of a correction for temperature, according to the method referred to previously, which took into account the oil concentration in the extract. It should be noted that at least ten readings

TABLE 2.  
*Extracts: Rotations at 20°C. (200 mm. tube).*

OIL	SOLVENT	CONCENTRATION	DEGREES OF ARC	MINUTES OF ARC	DEGREES V.
		<i>per cent</i>			
Lemon I. .	Ordinary Alcohol . .	5	5.708	342.5	16.47
Lemon II. .	Ordinary Alcohol . .	5	5.698	341.8	16.44
Orange III. .	Ordinary Alcohol . .	5	9.039	542.3	26.08
Orange IV. .	Ordinary Alcohol . .	5	9.17	550.1	26.45
Orange V. .	Ordinary Alcohol . .	5	9.058	543.5	26.14
Lemon I. .	Absolute Alcohol . .	5	5.706	342.4	16.46
Orange III. .	Absolute Alcohol . .	5	9.046	542.8	26.10
Lemon I. .	Ordinary Alcohol . .	1	1.124	67.6	3.24
Orange III. .	Ordinary Alcohol . .	1	1.780	106.9	5.14
Lemon I. .	Absolute Alcohol . .	1	1.134	68.0	3.27
Orange III. .	Absolute Alcohol . .	1	1.757	105.4	5.06

<sup>1</sup> Gildemeister and Hoffmann. *The Volatile Oils*, translated by Kremers, 1900, pp. 465, 471; Landolt. *The Optical Rotating Power of Organic Substances and Its Practical Applications*, translated by Long, 1902, p. 661.

were made in the case of each extract and that in only one case did the highest reading differ from the lowest by an amount greater than 2' of arc when calculated to the 200 mm. basis; in most cases it was less than 1'. The exception was the extract of California orange oil, examined in a 200 mm. tube: here the extreme variation was 3'.

The differences that exist in the figures shown in Table 2 seem to be due entirely to errors made in the preparation of the samples or in the polarimetric observations, and not to the nature of the solvent. Naturally, greater variations are to be expected in the case of the 1 per cent extracts, since the probable error in the preparation of the sample and in the polarimetric readings is about five times greater than for the 5 per cent extracts.

It will be noted that the rotations of the extracts are not so great, respectively, as would be anticipated from their concentration, assuming that the solvent acted, from the standpoint of rotation, simply as a diluent. This is shown in Table 3.

TABLE 3.

*Rotations of oils and of extracts compared (Basis: 100 mm. column).*

A—SOLUTIONS IN ORDINARY ALCOHOL.					
OIL	OIL	5 PER CENT EXTRACT	RATIO	1 PER CENT EXTRACT	RATIO
	<i>degrees</i>	<i>degrees</i>		<i>degrees</i>	
Lemon I . . .	61.566	2.854	20: 0.927	0.562	100: 0.913
Lemon II . . .	61.884	2.849	20: 0.921		
Orange III . . .	96.80	4.520	20: 0.934	0.890	100: 0.919
Orange IV . . .	98.115	4.585	20: 0.935		
Orange V . . .	97.975	4.529	20: 0.924		
B.—SOLUTIONS IN ABSOLUTE ALCOHOL					
Lemon I . . .	61.566	2.853	20: 0.927	0.567	100: 0.920
Orange III . . .	96.80	4.523	20: 0.934	0.877	100: 0.906

According to the figures given in Table 3, the rotatory power of these oils when in solution in alcohol—whether ordinary or absolute—is only about 92–93 per cent of what it is in the undissolved state.

NOTE.—In the work of Gnadinger this phenomenon has been studied in some detail. Gnadinger found that, in the case of a certain lemon oil, the rotatory power of a 10 per cent solution (at 25°C.) varied from 92 per cent to 104 per cent of that calculated from the known volume of oil present, according to the nature of the solvent used. In the case of the fourteen 5 per cent alcoholic extracts of lemon oil examined by Gnadinger the ratios found ranged from 0.915 to 0.949, with an average of 0.932; those for the three 5 per cent sweet orange extracts averaged 0.936.

It is conceivable that in the formation, say, of one of these 5 per cent solutions, such volume changes might take place in the oil, in the alcohol, or in both that the product would not consist of nineteen volumes of alcohol mixed with one volume of oil to form twenty volumes of extract. In such a case the specific gravity of the extract would vary more or less from the figure obtained by calculation, on the assumption that no volume change takes place when the ingredients are mixed. To test this point, a comparison was made of the specific gravities of two of the oils, of the ordinary alcohol, of the absolute alcohol, and of the 5 per cent extracts made from them. These determinations were made at 15°/15° in air, since that is the temperature usually employed in essential oil work. The 10 cc. pycnometer was used for this work; its capacity was found to be just under 9.5 cc. at 15°C.

TABLE 4.  
*Comparison of specific gravities made at 15°/15° in air.*

DETERMINATIONS	WATER	LEMON OIL I	ORANGE OIL III	ORDINARY ALCOHOL	ABSOLUTE ALCOHOL
Weight (in air) of pycnometer contents (grams)	9.3815	8.0508	7.9776	7.6404	7.4485
Specific gravity 15°/15°	1.0000	0.8582	0.8504	0.81441	0.79395
	5 PER CENT LEMON EXTRACT		5 PER CENT ORANGE EXTRACT		
	Ordinary Alcohol	Absolute Alcohol	Ordinary Alcohol	Absolute Alcohol	
Weight (in air) of pycnometer contents (grams)	7.6653	7.4783	7.6596	7.4747	
Specific gravity 15°/15°, found	0.81706	0.7971	0.8164	0.79675	
Specific gravity 15°/15°, calculated	0.8166	0.79716	0.8162	0.79677	

The "calculated" specific gravities given in Table 4 are, of course, based on the assumption that one volume of oil added to nineteen volumes of alcohol yields twenty volumes of extract. The agreement between the "found" and the "calculated" results would seem to show that no appreciable volume change takes place when the oil and alcohol are mixed—at any rate in these proportions. Hence the decrease in the rotatory power of these oils on going into alcoholic solution would appear to be chargeable to some intrinsic property of alcohol. That alcohol as a solvent does change the rotatory power of some substances, both in direction and in quantity, is well known<sup>1</sup>.

<sup>1</sup> See, e. g., Browne: Handbook of Sugar Analysis, 1912, pp. 181-2.

# FACTORS TO BE USED IN CALCULATING THE OIL CONCENTRATION FROM THE ROTATION PRODUCED BY THE EXTRACT.

It is well known that samples of lemon oil differ in rotatory power and in other characteristics according to source, method of preparation, age, method of storage, etc. The same is true of samples of sweet orange oil. Gildemeister and Hoffmann state that the usual limits are as follows:

	LEMON OIL	SWEET ORANGE OIL
Rotation at 20° (100 mm. tube)	+ 60° to 64°	+ 96° to 98°
Specific gravity: 15°/15°	0.858 - 0.861	0.848 - 0.852

In the case of lemon oil, according to these authors, the rotation is at times as low as + 59° and at times as high as + 67°. The rotations found in the case of the oils herein discussed (61.566° and 61.884°, in the case of lemon oil; 96.80°, 98.115°, and 97.975°, in the case of orange oil), and their specific gravities (0.8582 for Lemon Oil I and 0.8504 for Orange Oil III), would seem to indicate that the samples used were, on the whole, typical. If this is assumed to be so, the factors for finding percentage concentration from rotation would be as shown in Table 5.

TABLE 5.

*Rotation produced by 1 per cent by volume of oil at 20°C. (200 mm. tube)*

EXTRACTS	DEGREES OF ARC	MINUTES OF ARC	DEGREES VENTZKE
Lemon I extract (5 per cent; ordinary alcohol)	1.141	68.5	3.294
Lemon I extract (5 per cent; absolute alcohol)	1.141	68.5	3.292
Lemon II extract (5 per cent; ordinary alcohol)	1.140	68.4	3.288
Orange III extract (5 per cent; ordinary alcohol)	1.808	108.5	5.216
Orange III extract (5 per cent; absolute alcohol)	1.809	108.6	5.220
Orange IV extract (5 per cent; ordinary alcohol)	1.834	110.0	5.290
Orange V extract (5 per cent; ordinary alcohol)	1.812	108.7	5.228

Hence, to find the approximate percentage of oil by volume in an extract of lemon or of sweet orange the rotation at 20°C. in a 200 mm. tube should be divided by the appropriate figure given below.

	DEGREES OF ARC	MINUTES OF ARC	DEGREES VENTZKE
Lemon extract.	1.14	68.5	3.29
Sweet orange extract	1.81	109	5.23

But a study of Gnadinger's results and of those obtained by other observers, which have been brought to the writer's attention since the foregoing paragraphs were written, indicates that the average rotation of lemon oils is not so great as that here assumed (61.7°). Thus, Gnadinger's fourteen samples yielded an average of 60.4° of arc. His three samples of orange oil, on the other hand, showed a somewhat higher average rotation (97.8°) than did those listed above (97.63°).

Unpublished data collected by H. D. Poore for the U. S. Department of Agriculture in 1920 have to do with nearly 300 samples of lemon oil; of these the rotation in the case of 281, believed to be unadulterated<sup>1</sup>, averages 60.04° of arc. If this figure be combined with the figures for Gnadinger's fourteen samples and the two obtained by the writer, an average of 60.07° is obtained for the entire group of 297.

Poore's data for sweet orange oils have to do with 28 samples collected in Sicily and 27 samples taken from lots imported at New York. The average rotation of the former group is given as 97.45°; of the latter group as 95.50°. One is led to suspect—bearing in mind the limits given by Gildemeister and Hoffmann and the results found by Gnadinger and by the writer—that Poore's second group contained samples of bitter orange oil, or samples consisting of mixtures of the two oils. However, accepting all the data given as reliable, a total of 61 samples with an average rotation of 96.61° is found.

In Gnadinger's fourteen 5 per cent lemon extracts the rotation is, on the average, 4.66 per cent of that of the oil; in the three cases recorded in this paper it is 4.625 per cent. Using the figure 4.65 per cent, the rotation produced by a 200 mm. column of a 5 per cent lemon extract at 20°C. would be, on the average, 5.587° of arc, or 16.118° V. Similarly, in Gnadinger's three 5 per cent sweet orange extracts, the rotation is, on the average, 4.68 per cent of that of the oil; in the four extracts recorded by the writer 4.659 per cent. Assuming the figure 4.67 per cent, the rotation produced by a 200 mm. column of a 5 per cent sweet orange extract at 20°C. would be, on the average, 9.023° of arc, or 26.034° V.

Hence, to find the approximate percentage of oil by volume in an extract of lemon or of sweet orange, the rotation at 20°C. in a 200 mm. tube should be divided by the appropriate figure, as follows:

	DEGREES OF ARC	MINUTES OF ARC	DEGREES VENTZKE
Lemon extract . . . . .	1.12	67.0	3.22
Sweet orange extract. . . . .	1.81	108.3	5.21

<sup>1</sup> Most of these samples were collected by E. M. Chace and A. S. Cheney. See Chace: U. S. Dept. Agr. Bur. Chem. Cir. 46.







WILLIAM ALPHONSO WITHERS, 1864—1924

## WILLIAM ALPHONSO WITHERS

The "News and Observer" of Raleigh, N. C., carried, on Saturday morning, June 21, 1924, an account of the sudden passing on June 20th of Dr. W. A. Withers, who was for many years a member of the Association of Official Agricultural Chemists, and a past president 1909-1910. Angina pectoris was assigned as the cause of his death.

William Alphonso Withers was born May 31, 1864, in Riverview, N. C., near Davidson College. He and I entered the sub-freshman class at Davidson College together in September, 1878, and we graduated in the class of 1883. I always found Withers a good, reliable friend, and I remember very pleasantly having been entertained at his home in Riverview. Withers pursued a postgraduate course at Davidson College and received the degree of A.M. in 1885; he also took a postgraduate course at Cornell University, holding a fellowship from 1888 to 1890. In 1917, the degree of Sc.D. was conferred upon him by his Alma Mater. From 1884 to 1888 Withers was an assistant chemist; from 1897 to 1921, chemist, and from 1897 to 1899, acting director of the North Carolina Agricultural Experiment Station.

Dr. Withers was professor of chemistry at the A. and M. College of North Carolina, now known as the North Carolina State College, from the founding of that institution in 1889 until the time of his death on June 20, 1924. He was vice-president of the institution in 1916 and director of the summer school in 1917. He served as teacher of chemistry and physics at Peace Institute, Raleigh, N. C., from 1890 to 1893.

Dr. Withers served as statistical agent in the Department of Agriculture from 1895 to 1902 and from 1905 to 1915; State chemist of North Carolina from 1897 to 1898; member of the Executive Committee, Pure Food and Drug Congress, 1898; member of the American Association for the Advancement of Science; president of the North Carolina section of the American Chemical Society, 1901; member of the Society of Chemical Industry; chairman of the Committee on Pure Food Legislation in the Association of Agricultural Colleges and Experiment Stations from 1899 to 1905; member of the Society for Promotion of Agricultural Science; and president of the North Carolina Academy of Sciences in 1917.

During the war Dr. Withers was a member of the North Carolina Council of Defense, a member of the Wake County Food Administration Board, and also a member of the Executive Committee of the local Red Cross Chapter.

In civic life Dr. Withers devoted much time to Chamber of Commerce work, serving a number of terms on its Board of Directors, and at the time of his death he was president of that organization. He was also a member of the Rotary Club of Raleigh and a former president.

Dr. Withers was a Mason and rose high in the order. He was Grand Commander of Knights Templar of North Carolina in 1896 and Grand High Priest of Royal Arch Masons in 1897.

The religious activities of Dr. Withers included deep interest in church and Sunday school work, the Young Men's Christian Association, and the State Sunday School Organization. He was an elder in the Presbyterian Church and a former superintendent of Sunday school and was at one time president of the Young Men's Christian Association. He also served on the Executive Committee of the North Carolina Sunday School Association.

Judging from my own experience, I venture to say that a large part of Dr. Withers' time was taken up by his academic and administrative duties. He found time, however, for studies on food adulteration, nitrification, and cottonseed. He was the author of the 1899 Pure Food Law of North Carolina. In collaboration with F. A. Carruth, he discovered, in 1915, the toxic principle of cottonseed.

The following partial list of publications by Dr. Withers will give some idea of the scope of his work:

"A Modification of the Diphenylamine Test for Nitrous and Nitric Acids"—Withers and Ray—*Journal of The American Chemical Society*, Volume 33, No. 5, pages 708-711, May 1911.

"A Conductivity Study of the Reaction Between Calcium Nitrate and Dipotassium Phosphate in Dilute Solutions"—*Journal of The American Chemical Society*, Volume 37, No. 5, pages 1093-1105, May 1915.

*Studies in Soil Bacteriology:*

"The Rate of Nitrification of Some Fertilizers"—Withers and Fraps—*Proceedings of the Association of Official Agricultural Chemists*, pages 25-27, 1900; and *Journal of The American Chemical Society*, Volume 23, No. 5, pages 318-326, May 1901.

"The Nitrification of Ammonium Sulphate and Cottonseed Meal in Different Soils"—Withers and Fraps—*Proceedings of the Association of Official Agricultural Chemists*, pages 36-40, 1901.

"Nitrifying Power of Typical North Carolina Soils"—Report of Chemist, North Carolina Agricultural Experiment Station, 1902-1903; also published under the title of "Nitrification in Different Soils"—*Journal of The American Chemical Society*, Volume 24, No. 6, pages 528-534, June 1902.

"Methods for Determination of the Nitrifying and Ammonifying Powers of Soils"—Withers and Stevens—*Proceedings of the Association of Official Agricultural Chemists*, pages 34-38, 1909.

*Studies in Cottonseed Meal Intoxication:*

"Studies in the Toxicity of Cottonseed Meal"—Withers and Ray—*Proceedings of the Society for the Promotion of Agricultural Science*, pages 19-21, 1912.

I. "Pyrophosphoric Acid"—Withers and Ray—*Journal of Biological Chemistry*, Volume 14, No. 2, pages 53-58, March 1913.

"A Method for the Removal of the Toxic Properties from Cottonseed Meal"—A Preliminary Report—Withers and Ray—*Science*, n. s., Volume 36, No. 914, pages 31-32, 1912.

"Studies of Cottonseed Meal Toxicity. II. Iron as an Antidote"—Withers and Brewster with the collaboration of R. S. Curtis, G. A. Roberts, L. F. Williams, and J. W. Nowell—*Journal of Biological Chemistry*, Volume 15, No. 1, pages 161-166, July 1913.

"Gossypol, The Toxic Substance in Cottonseed Meal"—Withers and Carruth—*Journal of Agricultural Research*, Volume 5, No. 7, pages 261-288, 1915.

"Iron as an Antidote to Cottonseed Meal Injury"—Withers and Carruth—*Journal of Biological Chemistry*, Volume 32, No. 2, pages 245-257, November 1917.

"Gossypol, the Toxic Substance in Cottonseed"—Withers and Carruth—*Journal of Agricultural Research*, Volume 12, No. 2, pages 83-102, January 14, 1918.

"Comparative Toxicity of Cottonseed Products"—Withers and Carruth—*Journal of Agricultural Research*, Volume 14, No. 10, September 2, 1918.

Dr. Withers was connected with the Association of Official Agricultural Chemists for many years and served it in many important capacities. According to the sketch of the association presented by Dr. H. W. Wiley, then secretary of the association, and published in the proceedings of the sixteenth annual convention, Dr. Withers had attended eight meetings up to and including the fifteenth convention. A search of the proceedings of the association shows that Dr. Withers attended twenty-six meetings out of the forty that have been held.

In the absence of the president and vice-president, Dr. Withers served as acting president of the association at the twenty-first annual convention held at St. Louis, Mo., in 1904. The proceedings of this meeting show that he made a brief talk to the Fertilizer Manufacturers' Association, our association having been invited to attend their meetings. At the twenty-fifth annual convention (1908) Dr. Withers was elected vice-president of the association, and at the twenty-sixth convention he served as vice-president and was elected president, in which capacity he served at the twenty-seventh convention (1910) and delivered the presidential address on the "Teaching of Chemistry in American Colleges".

At the twenty-second annual convention (1905) Dr. Withers was appointed a member of Committee A on Recommendations of Referees. The proceedings of the twenty-seventh annual convention (1910) show in the list of special committees that Dr. Withers was appointed chairman of the Special Committee on Journal of Agricultural Research. At the

twenty-eighth annual convention (1911) he served as acting chairman of the Committee on Nominations. At the twenty-ninth convention (1912) he was appointed on the Committee on Editing Methods of Analysis and also served as chairman of the Committee on Nominations. He served on the Committee on Editing Methods of Analysis at the thirtieth to the thirty-third conventions, inclusive. Dr. Withers did not attend the conventions from the thirty-fifth to the thirty-eighth, inclusive, but was present at the thirty-ninth convention (1923).

At the seventeenth convention (1900) Withers and Fraps presented a paper on "The Rate of Nitrification of Certain Fertilizers". Again at the eighteenth convention (1901) the same authors presented the report on soils, entitled "The Nitrification of Ammonium Sulphate and Cottonseed Meal in Different Soils". At the twenty-sixth convention (1909) Withers and Stevens presented a paper on "Methods for the Determination of the Nitrifying and Ammonifying Powers of Soils", and at the thirty-first convention Withers and Carruth presented a paper on "Gossypol—A Toxic Substance in Cottonseed".

As a man Withers was characterized by modesty and integrity. He was a true and loyal friend, a lover of home and family, and from early youth an earnest, conscientious Christian. His sympathies and interests were broad, as shown by his diligence as teacher and research worker, his keen and active interest in civic affairs, and his devotion to the things of the spirit. He was twice married and leaves a widow with three children, two sons and a daughter, and a son by his first wife, to mourn his loss, and to whom he has left the precious legacy of a life well spent, rich in service to God and his fellowmen. With it all he found time to make discoveries in the science to which he devoted his life.

William Alphonso Withers passed away in the prime of life and left the world better for his having lived in it.

R. N. BRACKETT.



# PROCEEDINGS OF THE FORTIETH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1924.

The fortieth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 20-22, 1924.

The meeting was called to order by the President, R. E. Doolittle, Food and Drug Inspection Station, Chicago, Ill., on the morning of October 20, 1924.

## OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING OCTOBER, 1925.

### *Honorary President.*

HARVEY W. WILEY, Woodward Building, Washington, D. C.

### *President.*

C. A. BROWNE, Bureau of Chemistry, Washington, D. C.

### *Vice President.*

H. D. HASKINS, Amherst, Mass.

### *Secretary-Treasurer.*

W. W. SKINNER, Bureau of Chemistry, Washington, D. C.

### *Additional Members of the Executive Committee.*

W. W. RANDALL, Baltimore, Md.

W. H. MACINTIRE, Knoxville, Tenn.

### PERMANENT COMMITTEES.

#### *Committee to Cooperate with Other Committees on Food Definitions.*

JULIUS HORTVET (State Dairy and Food Commission, St. Paul, Minn.), *Chairman.*

C. D. HOWARD, Concord, N. H.

E. M. BAILEY, New Haven, Conn.

*Committee to Cooperate in Revision of the U. S. Pharmacopeia.*L. F. KEBLER (Bureau of Chemistry, Washington, D. C.), *Chairman.*

H. C. LYTGOE.

A. R. BLISS.

H. C. FULLER.

J. M. DORAN.

*Recommendations of Referees.*

(Figures in parentheses refer to year in which appointment expires.)

SUB-COMMITTEE A: B. B. Ross (1926), (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman*; J. W. Kellogg (1928); A. G. McCall (1930). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides; soils and liming materials (reaction values of soils, liming materials); feeding stuffs (linseed meal, stock feed adulteration, determination of moisture); sugars and sugar products (polariscopic methods; chemical methods for reducing sugars; drying, densimetric, and refractometric methods; honey; maple products; starch conversion products); fertilizers (nitrogen, potash, phosphoric acid); plants (sulfur and potash).]

SUB-COMMITTEE B: H. C. Lythgoe (1926), (Department of Public Health, Boston, Mass.), *Chairman*; A. G. Murray (1928); E. M. Bailey (1930). [Spices and other condiments, limits of accuracy in the determination of small amounts of alcohol, testing chemical reagents, naval stores (turpentine), drugs (acetylsalicylic acid, alcohol in drugs, arsenicals, camphor and monobromated camphor, chaulmoogra oil, chloramine-T products, chloroform and chloral hydrate, ipecac alkaloids, radio activity in drugs and water, laxatives and bitter tonics, mercurials, pyramidon, separation of quinine and strychnine, silver proteinates, nitroglycerin, apomorphine, santonin, ether).]

SUB-COMMITTEE C: R. E. Doolittle (1926), (Transportation Building, Chicago, Ill.), *Chairman*; C. F. Whitney (1928); H. A. Lepper (1930). [Dairy products (moisture in cheese, malted milk and dried milk, ice cream), fats and oils, baking powders and baking chemicals (fluorides in baking powder), eggs and egg products (total solids determination and unsaponifiable matter, ash and water-soluble protein-nitrogen precipitable by 40% alcohol and sampling of flaked eggs, zinc in dried eggs, acidity of fat and acid-soluble phosphoric acid), food preservatives, coloring matters in foods, metals in foods, fruits and fruit products (pectin in jams, jellies, and preserves; fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins, determination of sugar), gelatin, cereal foods (moisture in flour, ash in flour, chlorine in bleached flour, glutenin in flour, methods for sampling flour, methods for bread analysis), cacao products (microscopical methods, crude fiber, cacao butter).]

*Board of Editors.*

R. W. BALCOM (Box 290, Pennsylvania Avenue Station, Washington, D. C.), *Chairman* (1926).

R. B. DEEMER (1925).

R. E. DOOLITTLE (1928).

W. F. HAND (1927).

H. D. HASKINS (1929).

MARIAN E. LAPP, *Associate Editor.**Committee on Editing Methods of Analysis.*R. E. DOOLITTLE (Transportation Building, Chicago, Ill.), *Chairman.*

B. B. ROSS.

J. W. SALE.

A. J. PATTEN.

G. W. HOOVER.

W. H. MACINTIRE.

## SPECIAL COMMITTEES.

*Committee on Definitions of Terms and Interpretation of Results on Fertilizers.*

H. D. HASKINS (Agricultural Experiment Station, Amherst, Mass.), *Chairman.*

J. W. KELLOGG.

G. S. FRAPS.

E. G. PROULX.

R. N. BRACKETT.

*Committee on Revision of Methods of Soil Analysis.*

W. H. MACINTIRE (University of Tennessee, Knoxville, Tenn.), *Chairman.*

A. W. BLAIR.

J. A. BIZZELL.

R. STEWART.

A. G. MCCALL.

*Committee on Quartz-Plate Standardization and Normal Weight.*

FREDERICK BATES (Bureau of Standards, Washington, D. C.), *Chairman.*

C. A. BROWNE.

F. W. ZERBAN.

*Representatives on the Board of Governors of the Crop Protection Institute of the National Research Council.*

B. L. HARTWELL, Kingston, R. I.

H. J. PATTERSON, College Park, Md.

*Committee on Sampling.*

F. C. BLANCK (Bureau of Chemistry, Washington, D. C.), *Chairman.*

F. C. Blanck: *Foods.*

J. W. Kellogg: *Foods and Feeding Stuffs.*

A. E. Paul: *Drugs.*

A. G. McCall: *Soils and Liming Materials.*

C. C. McDonnell: *Insecticides and Fungicides.*

R. N. Brackett: *Fertilizers.*

F. W. Zerban: *Saccharine Products.*

R. W. Frey: *Tanning Materials and Leathers.*

J. W. Sale: *Water.*

A. J. Patten: *Plants.*

*Committee to Consider the Advisability of Studying Methods for the Analysis of Paint.*

W. F. HAND (Agricultural and Mechanical College, Agricultural College, Miss.),  
*Chairman.*

W. T. PEARCE.

J. W. KELLOGG.

*Committee on Bibliography.*

W. W. SKINNER (Bureau of Chemistry, Washington, D. C.), *Chairman.*

G. S. FRAPS.

H. D. HASKINS.

F. P. VEITCH.

W. W. RANDALL.



REFEREES AND ASSOCIATE REFEREES.

FERTILIZERS:

*General referee:* G. S. Fraps, College Station, Texas.

NITROGEN:

*Associate referee:* A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

POTASH:

*Associate referee:* A. P. Kerr, Agricultural Experiment Station, Baton Rouge, La.

PHOSPHORIC ACID:

*Associate referee:* W. H. Ross, Bureau of Soils, Washington, D. C.

PLANTS:

*General referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

SULFUR AND POTASH:

*Associate referee:* W. L. Latshaw, Agricultural Experiment Station, Manhattan, Kans.

SOILS AND LIMING MATERIALS:

*General referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

REACTION VALUES OF SOILS:

*Associate referee:* P. S. Burgess, Agricultural Experiment Station, Tucson, Ariz.

LIMING MATERIALS:

*Associate referee:* W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

TANNING MATERIALS AND LEATHERS:

*General referee:* R. W. Frey, Bureau of Chemistry, Washington, D. C.

INSECTICIDES AND FUNGICIDES:

*General referee:* J. J. T. Graham, Bureau of Chemistry, Washington, D. C.

WATERS, BRINE, AND SALT:

*General referee:* C. H. Badger, Bureau of Chemistry, Washington, D. C.

FEEDING STUFFS:

*General referee:* L. E. Bopst, State Control Department, College Park, Md.

LINSEED MEAL:

*Associate referee:* M. R. Coe, Bureau of Chemistry, Washington, D. C.

STOCK FEED ADULTERATION:

*Associate referee:* H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

DETERMINATION OF MOISTURE:

*Associate referee:* W. F. Sterling, Bureau of Chemistry, Washington, D. C.

SUGARS AND SUGAR PRODUCTS:

*General referee:* H. S. Paine, Bureau of Chemistry, Washington, D. C.

POLARISCOPIC METHODS:

*Associate referee:* F. W. Zerban, 80 South Street, New York, N. Y.

**CHEMICAL METHODS FOR REDUCING SUGAR:**

*Associate referee:* R. F. Jackson, Bureau of Standards, Washington, D. C.

**DRYING, DENSIMETRIC AND REFRACTOMETRIC METHODS:**

*Associate referee:* Max Schneller, 111 Wall Street, New York, N. Y.

**HONEY:**

*Associate referee:* William Seaman, Food and Drug Station, New York, N. Y.

**MAPLE PRODUCTS:**

*Associate referee:* H. M. Lancaster, 317 Queen Street, Ottawa, Canada.

**STARCH CONVERSION PRODUCTS:**

*Associate referee:* F. W. Reynolds, Bureau of Chemistry, Washington, D. C.

**FOOD PRESERVATIVES:**

*General referee:* W. W. Randall, State Department of Health, Baltimore, Md.

**COLORING MATTERS IN FOODS:**

*General referee:* C. F. Jablonski, 641 Washington Street, New York, N. Y.

**METALS IN FOODS:**

*General referee:* B. G. Hartmann, Bureau of Chemistry, Washington, D. C.

**FRUITS AND FRUIT PRODUCTS:**

*General referee:* P. L. Gowen, Bureau of Chemistry, Washington, D. C.

**PECTIN IN JAMS, JELLIES, AND PRESERVES:**

*Associate referee:* H. J. Wichmann, U. S. Food and Drug Inspection Station, Denver, Colo.

**FRUIT ACIDS:**

*Associate referee:* E. K. Nelson, Bureau of Chemistry, Washington, D. C.

**CANNED FOODS:**

*General referee:* To be appointed.

**CEREAL FOODS:**

*General referee:* Raymond Hertwig, Bureau of Chemistry, Washington, D. C.

**MOISTURE IN FLOUR:**

*Associate referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**ASH IN FLOUR:**

*Associate referee:* C. E. Mangels, Agricultural Experiment Station, Agricultural College, N. Dak.

**CHLORINE IN BLEACHED FLOUR:**

*Associate referee:* Armin Seidenberg, Department of Health, New York, N. Y.

**GLUTENIN IN FLOUR:**

*Associate referee:* M. J. Blish, University of Nebraska, Lincoln, Nebr.

**METHODS FOR SAMPLING FLOUR:**

*Associate referee:* H. Runkel, Food and Drug Inspection Station, Minneapolis, Minn.

**METHODS FOR THE EXAMINATION OF BREAD:**

*Associate referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

METHODS FOR FAT (ACID HYDROLYSIS METHOD), LIPOIDS AND LIPOID-PHOSPHORIC ACID, WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITATION BY 40 PER CENT ALCOHOL, AND UNSAPONIFIABLE MATTER:

*Associate referee:* Samuel Alfend, Food and Drug Inspection Station, St. Louis, Mo.

HYDROGEN ION CONCENTRATION OF FLOUR:

*Associate referee:* C. H. Bailey, 565 W. Washington St., Chicago, Ill.

STARCH AND DIASTATIC VALUE OF FLOUR:

*Associate referee:* To be appointed.

DETERMINATION OF SPECIFIC GRAVITY AND ALCOHOL:

*General referee:* R. N. Hann, Bureau of Chemistry, Washington, D. C.

VINEGARS:

*General referee:* J. O. Clarke, Food and Drug Inspection Station, Savannah, Ga.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:

*General referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

MEAT AND MEAT PRODUCTS:

*General referee:* R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:

*Associate referee:* W. W. Ritchie, University of Missouri, Columbia, Mo.

DETERMINATION OF SUGAR:

*Associate referee:* To be appointed.

GELATIN:

*General referee:* E. H. Berry, 1625 Transportation Building, Chicago, Ill.

EGGS AND EGG PRODUCTS:

*General referee:* Raymond Hertwig, Bureau of Chemistry, Washington, D. C.

TOTAL SOLIDS DETERMINATION AND UNSAPONIFIABLE MATTER:

*Associate referee:* M. L. Hitchcock, 1625 Transportation Building, Chicago, Ill.

ASH AND WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL AND SAMPLING OF FLAKED EGGS:

*Associate referee:* J. C. Palmer, Room 33, U. S. Appraiser's Stores, Sansome and Washington Streets, San Francisco, Calif.

ZINC IN DRIED EGGS:

*Associate referee:* W. E. Kirby, 641 Washington Street, New York, N. Y.

ACIDITY OF FAT AND ACID-SOLUBLE PHOSPHORIC ACID:

*Associate referee:* H. I. Macomber, 641 Washington Street, New York, N. Y.

DAIRY PRODUCTS:

*General referee:* Julius Hortvet, Dairy and Food Commission, St. Paul, Minn.

MOISTURE IN CHEESE:

*Associate referee:* L. C. Mitchell, Room 204 Old Custom House, St. Louis, Mo.

ICE CREAM:

*Associate referee:* Jacob Moyer, Agricultural College, Fargo, N. Dak.

MALTED MILK AND DRIED MILK:

*Associate referee:* J. T. Keister, Bureau of Chemistry, Washington, D. C.

**FATS AND OILS:**

*General referee:* G. S. Jamieson, Bureau of Chemistry, Washington, D. C.

**SPICES AND OTHER CONDIMENTS:**

*General referee:* W. C. Geagley, Department of Agriculture, Lansing, Mich.

**CACAO PRODUCTS:**

*General referee:* E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

**MICROSCOPICAL METHODS:**

*Associate referee:* V. A. Pease, Bureau of Chemistry, Washington, D. C.

**CRUDE FIBER:**

*Associate referee:* E. R. Miller, U. S. Appraiser's Stores, New York, N. Y.

**CACAO BUTTER:**

*Associate referee:* W. F. Baughman, Bureau of Chemistry, Washington, D. C.

**BAKING POWDERS AND BAKING CHEMICALS:**

*General referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

**FLUORIDES IN BAKING POWDER:**

*Associate referee:* J. K. Morton, Bureau of Chemistry, Washington, D. C.

**TESTING CHEMICAL REAGENTS:**

*General referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**NAVAL STORES:**

*General referee:* F. P. Veitch, Bureau of Chemistry, Washington, D. C.

**TURPENTINE:**

*Associate referee:* V. E. Grotlisch, Bureau of Chemistry, Washington, D. C.

**DRUGS:**

*General referee:* A. E. Paul, 1625 Transportation Building, Chicago, Ill.

**ACETYSALICYLIC ACID:**

*Associate referee:* C. W. Harrison, Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.

**ALCOHOL IN DRUGS:**

*Associate referee:* E. V. Lynn, University of Washington. Seattle, Wash.

**ARSENICALS:**

*Associate referee:* C. K. Glycart, 1625 Transportation Building, Chicago, Ill.

**CAMPOR AND MONOBROMATED CAMPOR:**

*Associate referee:* E. O. Eaton, U. S. Appraiser's Stores, San Francisco, Calif.

**CHAULMOOGRA OIL:**

*Associate referee:* L. E. Warren, 535 N. Dearborn Street, Chicago, Ill.

**CHLORAMINE-T PRODUCTS:**

*Associate referee:* L. Jones, 1625 Transportation Building, Chicago, Ill.

**CHLOROFORM AND CHLORAL HYDRATE:**

*Associate referee:* H. O. Moraw, 1625 Transportation Building, Chicago, Ill.

**IPECAC ALKALOIDS:**

*Associate referee:* A. R. Bliss, jr., University of Tennessee, Memphis, Tenn.

**RADIO ACTIVITY IN DRUGS AND WATER:**

*Associate referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

**LAXATIVES AND BITTER TONICS:**

*Associate referee:* H. C. Fuller, Industrial Research Laboratories, Washington, D. C.

**MERCURIALS:**

*Associate referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**PYRAMIDON:**

*Associate referee:* William Rabak, 311 Federal Office Building, Minneapolis, Minn.

**SEPARATION OF QUININE AND STRYCHNINE:**

*Associate referee:* F. L. Elliot, U. S. Appraiser's Stores, Boston, Mass.

**SILVER PROTEINATES:**

*Associate referee:* M. L. Hitchcock, 1625 Transportation Building, Chicago, Ill.

**NITROGLYCERIN:**

*Associate referee:* A. W. Hanson, 1625 Transportation Building, Chicago, Ill.

**APOMORPHINE:**

*Associate referee:* C. K. Glycart, 1625 Transportation Building, Chicago, Ill.

**SANTONIN:**

*Associate referee:* Samuel Palkin, Bureau of Chemistry, Washington, D. C.

**ETHER:**

*Associate referee:* To be appointed.

**BIO-ASSAY OF DRUGS:**

*Associate referee:* To be appointed.

**MEMBERS AND VISITORS PRESENT, 1924 MEETING.**

Allen, Charles D., H. Kohnstamm & Co., Brooklyn, N. Y.

Allen, W. M., Raleigh, N. C.

Almy, L. H., Bureau of Chemistry, Washington, D. C.

Anderson, M. S., Bureau of Soils, Washington, D. C.

Arnold, Wm. S., The International Co., Baltimore, Md.

Atwater, C. G., 40 Rector St., New York City.

Bacon, Charles W., Bureau of Plant Industry, Washington, D. C.

Badger, C. H., Bureau of Chemistry, Washington, D. C.

Badollet, M. S., Bureau of Chemistry, Washington, D. C.

Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.

Bailey, L. H., Bureau of Chemistry, Washington, D. C.

Bainbridge, W. C., H. Kohnstamm & Co., Brooklyn, N. Y.

Baird, R. O., Fargo, N. Dak.

Baker, J. H., University of Maryland, College Park, Md.

Balcom, R. W., Bureau of Chemistry, Washington, D. C.

Barnes, Jesse Wright, Bureau of Chemistry, Washington, D. C.

Bates, Carleton, Milwaukee, Wis.

Bates, Frederick, Bureau of Standards, Washington, D. C.

Baughman, Walter F., Bureau of Chemistry, Washington, D. C.

- Beal, W. H., Office of Experiment Stations, Washington, D. C.  
Beyer, G. F., Bureau of Internal Revenue, Washington, D. C.  
Bidwell, G. L., Bureau of Chemistry, Washington, D. C.  
Bigelow, W. D., National Canners Association, Washington, D. C.  
Bliss, A. R., Jr., University of Tennessee, Memphis, Tenn.  
Bopst, L. E., College Park, Md.  
Bowen, C. V., 3026 Wisconsin Ave., Washington, D. C.  
Boyle, Martin, Bureau of Chemistry, Washington, D. C.  
Boyles, F. M., Baltimore, Md.  
Boynton, Alice A., Bureau of Chemistry, Washington, D. C.  
Bradbury, C. M., U. S. Dept. of Agriculture, Richmond, Va.  
Breckenridge, J. E., American Agricultural Chemical Co., Carteret, N. J.  
Bridges, Fred T., 203 Winter Ave., Atlanta, Ga.  
Brooke, Mary Minton, Corby Baking Co., Washington, D. C.  
Broughton, L. B., University of Maryland, College Park, Md.  
Brown, B. E., Department of Agriculture, Washington, D. C.  
Browne, C. A., Bureau of Chemistry, Washington, D. C.  
Browne, Louise McDanell, 2301 Connecticut Ave., Washington, D. C.  
Bubb, John C., 719 Ninth St., N. E., Washington, D. C.  
Buchanan, Ruth, Bureau of Chemistry, Washington, D. C.  
Burritt, Loren, 1933 Eighteenth St., N. W., Washington, D. C.  
Burroughs, Lillian C., 16 W. Saratoga St., Baltimore, Md.  
Butt, Charles A., International Agricultural Corporation, Atlanta, Ga.
- Capen, Ruth G., Bureau of Chemistry, Washington, D. C.  
Carpenter, F. B., Virginia-Carolina Chemical Co., Richmond, Va.  
Carroll, John I., 81 Fulton St., New York, N. Y.  
Cathcart, Chas. S., New Brunswick, N. J.  
Chesnut, V. K., Bureau of Chemistry, Washington, D. C.  
Clarke, J. O., Bureau of Chemistry, Savannah, Ga.  
Clarke, W. F., Bureau of Chemistry, Washington, D. C.  
Clevenger, J. F., Bureau of Chemistry, Washington, D. C.  
Coe, Mayne R., Bureau of Chemistry, Washington, D. C.  
Colson, Henry C., Jr., 215 Ninth St., S. W., Washington, D. C.  
Conrad, C. M., College Park, Md.  
Couch, James F., Bureau of Animal Industry, Washington, D. C.  
Cummings, Joseph A., Bureau of Chemistry, New York, N. Y.
- Davidson, J., Bureau of Chemistry, Washington, D. C.  
Davis, R. O. E., Bureau of Soils, Washington, D. C.  
Dawson, Paul R., Bureau of Plant Industry, Washington, D. C.  
Dedge, Ruby M., Bureau of Chemistry, Washington, D. C.  
Deemer, R. B., Bureau of Plant Industry, Washington, D. C.  
Dewar, E. S., Department of Agriculture, Raleigh, N. C.  
Dixon, H. B., 1030 Euclid St., N. W., Washington, D. C.  
Donaldson, E. C., Laurel, Md.  
Doolittle, R. E., Transportation Bldg. Chicago, Ill.  
Doran, J. M., Bureau of Internal Revenue, Washington, D. C.  
Dunbar, P. B., Bureau of Chemistry, Washington, D. C.  
Dunlap, F. L., 1457 Monadnock Block, Chicago, Ill.  
Duvall, Louise, Bureau of Chemistry, Washington, D. C.
- Edwards, P. W., Bureau of Chemistry, Washington, D. C.  
Ellett, W. B., Blacksburg, Va.

Emery, W. O., Bureau of Chemistry, Washington, D. C.  
English, Leah, 132 E. Court St., Ithaca, N. Y.  
Ethier, Laura S., Bureau of Chemistry, Washington, D. C.  
Evenson, O. L., Bureau of Chemistry, Washington, D. C.  
Everhart, Helen, Bureau of Chemistry, Washington, D. C.  
Ewing, C. O., United Drug Co., Boston, Mass.

Fellows, Harold C., Bureau of Agricultural Economics, Washington, D. C.  
Fiske, Augustus H., Rumford Chemical Works, Providence, R. I.  
Fletcher, C. C., Bureau of Soils, Washington, D. C.  
Fraps, G. S., College Station, Texas.  
Frisbie, W. S., Bureau of Chemistry, Washington, D. C.  
Fuller, H. C., 2201 New York Ave., Washington, D. C.

Gardiner, R. F., 1511 Twenty-second St., N. W., Washington, D. C.  
Gascoyne, W. G., 27 S. Gay St., Baltimore, Md.  
Geagley, W. C., State Food and Drug Department, Lansing, Mich.  
Gersdorff, Chas. E. F., Bureau of Chemistry, Washington, D. C.  
Gersdorff, W. A., 220 Tenth St., S. W., Washington, D. C.  
Gile, P. L., Bureau of Soils, Washington, D. C.  
Glycart, C. K., Transportation Bldg., Chicago, Ill.  
Gordon, Neil E., University of Maryland, College Park, Md.  
Gowen, P. L., Bureau of Chemistry, Washington, D. C.  
Graham, J. J. T., Bureau of Chemistry, Washington, D. C.  
Grattan, George E., Department of Agriculture, Ottawa, Ont., Canada.  
Grayson, Mary C., Bureau of Chemistry, Washington, D. C.  
Griffin, E. L., Bureau of Chemistry, Washington, D. C.  
Grotlisch, V. E., Bureau of Chemistry, Washington, D. C.

Halvorson, H. A., 215 Old Capitol Bldg., St. Paul, Minn.  
Hammond, H. S., Experimental Farm, Ottawa, Canada.  
Hanaway, Wm. L., Bureau of Chemistry, Washington, D. C.  
Hand, W. F., Agricultural and Mechanical College, Miss.  
Hanson, H. H., State Chemist, Dover, Del.  
Harding, T. Swann, Beltsville, Md.  
Harrison, Channing W., 218 Water St., Baltimore, Md.  
Hart, Gordon, State Chemist, Tallahassee, Fla.  
Hart, Leslie, Bureau of Chemistry, Washington, D. C.  
Hartmann, B. G., Bureau of Chemistry, Washington, D. C.  
Hausknecht, V. B., Bureau of Chemistry, Harrisburg, Pa.  
Haywood, J. K., Bureau of Chemistry, Washington, D. C.  
Henry, A. M., Bureau of Chemistry, Philadelphia, Pa.  
Hertwig, Raymond, Bureau of Chemistry, Washington, D. C.  
Hoagland, Ralph, Bureau of Animal Industry, Washington, D. C.  
Hollingshead, Robert S., Bureau of Foreign and Domestic Commerce, Washington, D. C.  
Holmes, Myron G., Bureau of Chemistry, Washington, D. C.  
Hoover, Geo. W., Bureau of Chemistry, Washington, D. C.  
Hopper, T. H., State Experiment Station, Fargo, N. D.  
Hortvet, Julius, Minnesota Dairy and Food Department, St. Paul, Minn.  
Howard, B. J., Bureau of Chemistry, Washington, D. C.  
Howard, Charles S., Geological Survey, Washington, D. C.  
Howe, H. E., Industrial & Engineering Chemistry, Washington, D. C.  
Howes, C. Clifton, Davison Chemical Co., Baltimore, Md.

Humphrey, H. J., Bureau of Chemistry, Buffalo, N. Y.  
Hurst, Lewis A., Bureau of Plant Industry, Washington, D. C.  
Huston, H. A., Kew Hall, Kew Gardens, New York.

Irwin, W. H., Swift & Co., Chicago, Ill.  
Isbell, Horace S., Riverdale, Md.

Jackson, Richard F., Bureau of Standards, Washington, D. C.  
Jamieson, George S., Bureau of Chemistry, Washington, D. C.  
Jarrell, T. D., Bureau of Chemistry, Washington, D. C.  
Jinkins, Rosewell, Bureau of Chemistry, Washington, D. C.  
Johnson, J. M., Twenty-fifth and E Sts., N. W., Washington, D. C.  
Jones, Chas. H., Agricultural Experiment Station, Burlington, Vt.  
Jones, D. Breese, Bureau of Chemistry, Washington, D. C.  
Jones, R. M., 1967 Biltmore St., Washington, D. C.

Kebler, L. F., Bureau of Chemistry, Washington, D. C.  
Keister, J. T., Bureau of Chemistry, Washington, D. C.  
Kellogg, James W., Department of Agriculture, Harrisburg, Pa.  
Kerr, A. P., Agricultural Experiment Station, Baton Rouge, La.  
Kerr, Robert H., Bureau of Animal Industry, Washington, D. C.  
Kunke, Wm. F., Bureau of Chemistry, Washington, D. C.

Lapp, Marian E., Bureau of Chemistry, Washington, D. C.  
Lathrop, C. P., National Preservers Association, Washington, D. C.  
Law, Thos. C., Law & Co., Atlanta, Ga.  
LeClerc, J. A., Department of Commerce, Washington, D. C.  
Leighty, Wilbur R., Bureau of Plant Industry, Washington, D. C.  
Lepper, Henry A., Bureau of Chemistry, Washington, D. C.  
Linder, Wm. V., Bureau of Internal Revenue, Washington, D. C.  
Lineweaver, A. N., F. S. Royster Guano Co., Norfolk, Va.  
Linton, F. B., Bureau of Chemistry, Washington, D. C.  
Lodge, F. S., Armour Fertilizer Works, Chicago, Ill.  
Loomis, Henry M., National Cannery Association, Washington, D. C.  
Lourie, Harry L., U. S. Tariff Commission, Washington, D. C.  
Lundstrom, Frank O., Bureau of Soils, Washington, D. C.  
Lynch, William D., Bureau of Chemistry, Washington, D. C.

McCall, A. G., College Park, Md.  
McDonnell, C. C., Bureau of Chemistry, Washington, D. C.  
McDonnell, H. B., College Park, Md.  
McKinney, Robert S., Bureau of Chemistry, Washington, D. C.  
McLaughlin, Laura, Bureau of Home Economics, Washington, D. C.  
MacIntire, W. H., University of Tennessee, Knoxville, Tenn.  
Macomber, Hugh I., Bureau of Chemistry, New York, N. Y.  
Magruder, E. W., F. S. Royster Guano Co., Norfolk, Va.  
Marciniak, Edw. S., Bureau of Chemistry, Washington, D. C.  
Martin, James B., 1408 Shepherd St., N. W., Washington, D. C.  
Mayer, Otto F., H. J. Heinz Co., Pittsburgh, Pa.  
Mehring, A. L., Bureau of Soils, Washington, D. C.  
Mehurin, R. M., 2031 F St., N. W., Washington, D. C.  
Merz, Albert R., Bureau of Soils, Washington, D. C.  
Miner, Carl S., Miner Laboratories, Chicago, Ill.  
Mitchell, A. S., Bureau of Chemistry, Washington, D. C.



- Mitchell, J. H., Agricultural Experiment Station, Clemson College, S. C.  
 Mitchell, L. C., 204 Old Custom House, St. Louis, Mo.  
 Mix, Miss A. E., Bureau of Chemistry, Washington, D. C.  
 Moeller, Otto, Bureau of Chemistry, Washington, D. C.  
 Moore, Mary D., Bureau of Chemistry, Washington, D. C.  
 Moraw, H. O., Bureau of Chemistry, Chicago, Ill.  
 Morgan, C. E., Bureau of Chemistry, Washington, D. C.  
 Morgan, Will J., 1725 Jackson St., N. E., Washington, D. C.  
 Morison, C. Brewster, American Institute of Baking, Chicago, Ill.  
 Morton, James K., Bureau of Chemistry, Washington, D. C.  
 Moulton, S. C., District of Columbia Health Department, Washington, D. C.  
 Munch, J. C., Bureau of Chemistry, Washington, D. C.  
 Murray, A. G., Bureau of Chemistry, Washington, D. C.  
 Myers, William A., Chilean Nitrate of Soda Co., New York, N. Y.  
  
 Nelson, E. K., Bureau of Chemistry, Washington, D. C.  
 Normington, Marian L., Bureau of Home Economics, Washington, D. C.  
  
 O'Brien, Alice B., Bureau of Chemistry, Washington, D. C.  
  
 Paine, H. S., Bureau of Chemistry, Washington, D. C.  
 Palkin, S., Bureau of Chemistry, Washington, D. C.  
 Palmore, Julian I., Bureau of Chemistry, Washington, D. C.  
 Parkins, John H., Norfolk Testing Laboratories, Inc., Norfolk, Va.  
 Parkinson, N. A., 1216 Delafield Place, Washington, D. C.  
 Patten, Andrew J., Agricultural Experiment Station, E. Lansing, Mich.  
 Patten, H. E., Silver Spring, Md.  
 Patterson, H. J., Agricultural Experiment Station, College Park, Md.  
 Paul, Arthur E., 1625 Transportation Building, Chicago, Ill.  
 Pease, V. A., Bureau of Chemistry, Washington, D. C.  
 Pingree, M. H., American Agricultural Chemical Co., Baltimore, Md.  
 Plummer, J. K., Tennessee Copper and Chemical Corporation, New York City.  
 Power, Frederick B., Bureau of Chemistry, Washington, D. C.  
 Pozen, M. A., Schwarz Laboratories, Inc., New York, N. Y.  
 Prince, Arthur L., Experiment Station, New Brunswick, N. J.  
 Proffitt, M. J., Bureau of Standards, Washington, D. C.  
 Proulx, E. G., State Chemist, W. Lafayette, Ind.  
  
 Randall, Wyatt W., State Department of Health, Baltimore, Md.  
 Reed, J. B., Bureau of Chemistry, Washington, D. C.  
 Reid, W. D., Swift & Co., Baltimore, Md.  
 Reindollar, Wm. F., 16 W. Saratoga St., Baltimore, Md.  
 Reinmuth, Otto, Catonsville, Md.  
 Rhodes, Leland B., State Department of Agriculture, Raleigh, N. C.  
 Riffenburg, H. B., U. S. Geological Survey, Washington, D. C.  
 Riley, H. N., H. J. Heinz Co., Pittsburgh, Pa.  
 Riley, J. G., Bureau of Internal Revenue, Washington, D. C.  
 Roberts, Chas. C., Arthur H. Thomas Co., Philadelphia, Pa.  
 Robinson, S. K., 408 Rush St., Chicago, Ill.  
 Ross, B. B., Polytechnic Institute, Auburn, Ala.  
 Ross, W. H., Bureau of Soils, Washington, D. C.  
 Rudnick, Paul, Armour & Co., Chicago, Ill.  
 Runk, C. R., University of Delaware, Newark, Del.

- Sale, J. W., Bureau of Chemistry, Washington, D. C.  
Sample, J. W., 706 Cedar St., Nashville, Tenn.  
Schertz, F. M., Bureau of Plant Industry, Washington, D. C.  
Schulze, Wilber H., 16 W. Saratoga St., Baltimore, Md.  
Schwartz, E. W., Bureau of Chemistry, Washington, D. C.  
Seidenberg, Armin, Department of Health, New York, N. Y.  
Shaver, Arthur, Bureau of Chemistry, Washington, D. C.  
Shepherd, Clara M., Bureau of Chemistry, Washington, D. C.  
Sherwood, S. F., Bureau of Plant Industry, Washington, D. C.  
Shingler, G. P., Food and Drug Inspection Station, Savannah, Ga.  
Shulenberg, F. W., Eimer and Amend, New York, N. Y.  
Skinner, J. J., Bureau of Plant Industry, Washington, D. C.  
Skinner, Laura A., Bureau of Chemistry, Washington, D. C.  
Skinner, W. W., Bureau of Chemistry, Washington, D. C.  
Smalley, H. R., Insurance Building, Washington, D. C.  
Smith, Janet K., Bureau of Chemistry, Washington, D. C.  
Smith, Joseph G., Bureau of Soils, Washington, D. C.  
Smith, Katherine A., Bureau of Chemistry, Washington, D. C.  
Smith, Philip H., Agricultural Experiment Station, Amherst, Mass.  
Smith, S. L., Department of Agriculture, Washington, D. C.  
Smith, W. C., Bureau of Chemistry, Washington, D. C.  
Smither, F. W., Bureau of Standards, Washington, D. C.  
Snyder, E. F., Bureau of Plant Industry, Washington, D. C.  
Snyder, Harry, Russell-Miller Milling Co., Minneapolis, Minn.  
Spencer, G. C., Bureau of Chemistry, Washington, D. C.  
Stengel, Arthur, Bureau of Chemistry, Washington, D. C.  
Stephenson, Chas. H., Bureau of Chemistry, Washington, D. C.  
Sterling, W. F., Bureau of Chemistry, Washington, D. C.  
Stevenson, A. E., National Canners Association, Washington, D. C.  
Stirlen, E. D., Yale University, New Haven, Conn.  
Stokes, W. E., Royal Baking Powder Co., Brooklyn, N. Y.  
Stowd, W. H., Department of Agriculture, Madison, Wis.  
Sullivan, A. L., 16 W. Saratoga St., Baltimore, Md.
- Taylor, A. E., Bureau of Chemistry, Washington, D. C.  
Taylor, J. N., Bureau of Animal Industry, Washington, D. C.  
Thomas, J. B., Chemical Supervisor, Baltimore, Md.  
Thornton, E. W., R. B. Davis Co., Hoboken, N. J.  
Tobey, E. R., Agricultural Experiment Station, Orono, Me.  
Toll, John D., 1010 Arch St., Philadelphia, Pa.  
Turner, Wm. A., Bureau of Plant Industry, Washington, D. C.  
Turrentine, J. W., Bureau of Soils, Washington, D. C.
- Valaer, Peter, Bureau of Internal Revenue, Washington, D. C.  
Van Slyke, L. L., Agricultural Experiment Station, Geneva, N. Y.  
Veitch, F. P., Bureau of Chemistry, Washington, D. C.  
von Czoernig, Carl, 1221 Arch St., Philadelphia, Pa.
- Walker, Percy H., Bureau of Standards, Washington, D. C.  
Walls, H. R., University of Maryland, College Park, Md.  
Walton, G. P., Bureau of Soils, Washington, D. C.  
Ward, Kyle, Bureau of Chemistry, Washington, D. C.  
Warren, Lewis E., American Medical Association, Chicago, Ill.

Waterman, H. C., Bureau of Chemistry, Washington, D. C.  
Watkins, Howard R., Bureau of Chemistry, Washington, D. C.  
Weber, F. C., Fleischmann Laboratories, New York, N. Y.  
Weems, J. B., Department of Agriculture, Richmond, Va.  
Wherry, E. H., Bureau of Chemistry, Washington, D. C.  
White, W. B., Department of Farms and Markets, Albany, N. Y.  
Whitney, C. F., Laboratory of Hygiene, Burlington, Vt.  
Wiley, Harvey W., Woodward Building, Washington, D. C.  
Wiley, Samuel W., Wiley & Co., Inc., Baltimore, Md.  
Williams, C. C., 1739 H St., N. W., Washington, D. C.  
Wilson, J. B., Bureau of Chemistry, Washington, D. C.  
Wilson, S. H., Room 130, State Capitol, Atlanta, Ga.  
Wilson, S. M., Baugh Chemical Co., Baltimore, Md.  
Woodbury, C. G., 1739 H St., N. W., Washington, D. C.  
Wright, C. D., Bureau of Chemistry, Washington, D. C.  
  
Zerban, F. W., N. Y. Sugar Trade Laboratory, New York City.

## PRESIDENT'S ADDRESS<sup>1</sup>.

### THE NEEDS OF OUR ASSOCIATION.

By R. E. DOOLITTLE (U. S. Food and Drug Inspection Station,  
Chicago, Ill.).

We meet under most auspicious circumstances this year, celebrating as we do the fortieth anniversary of our association and the eightieth anniversary of our beloved Honorary President, Dr. Harvey W. Wiley. Dr. Wiley tells me that he is writing his autobiography. I opine this will be interesting reading for future historians of the Association of Official Agricultural Chemists, as well as for others. Our association might also write an autobiography, and in that autobiography would appear more often and more conspicuous than anything else a recital of the deeds of faithful and efficient service rendered by Dr. Wiley. His life has been so closely interwoven with that of our association that we do not think of the one without bringing to mind the other. May it be many, many years before he writes the last chapter of that autobiography.

The only excuses I can offer for inflicting a presidential address upon you are the precedent established by 39 prior presidents and my earnest desire to in some way assist in perpetuating the traditions of enthusiasm and zeal which characterized the founders of our association and which have been handed down through these 40 years as a heritage worthy the best there is in us. We are justified in our pride for our association for reason of its accomplishments, but that pride should be coupled with a determination that the future shall bring forth even greater successes than those that have gone before. With this thought in mind, the question arises as to whether we, as an association, are doing all we can do to encourage participation in its work and to foster this spirit of enthusiasm and pride among our younger members. Correct methods of analysis are as essential to progress in agricultural chemistry as in any other line of chemistry. The quantity of work that is discredited or that has to be rejected for reason of faulty methods of analysis is appalling. Active participation in the work of the association is not only an inspiration and valuable training for the individual who plans and carries it out, but it also is of great benefit to the institution he represents through the confidence established in its output. But more than this, I believe some definite plan of procedure should be provided by which the chemist who takes an active part in the collaborative work of

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<sup>1</sup> Presented Tuesday morning, October 21st, as special order of business for 11 o'clock.

the association and by such work shows ability to assume more responsibility shall receive proper recognition by the association itself. This might be done by a systematic plan of promotion to the more important offices and committees. The association needs the enthusiasm and ideas of its younger members; at the same time, however, we should exercise care that we do not lose the advantage of the years of experience of those who have served long and faithfully. It appears in many instances that when a member of the association has once filled its principal offices he feels—or the association takes it for granted he feels—that he has done his part and may now rest on work done. These are the members best qualified to serve in the councils of the association, and the association should see that they are again brought into active participation in its work.

The most important problem that confronts the association as I study the situation is a proper coordination of its activities. Little difficulty is experienced in securing competent chemists to take charge of its investigative and collaborative studies, but a referee or associate referee, once selected, is left almost entirely to his own resources. Those of you who have served on Sub-committees A, B, and C well know how, in many instances, referees have taken up their work without consideration of the recommendations made or of the work done by former referees. Our proceedings are replete with reports on lines of work well begun but never finished. Again, retiring referees have made no recommendations for further work, and when new referees are in doubt as to what are the most important problems to take up, no one is designated to assist them. On the other hand, some referees have felt that they were restricted in their activities to the recommendations of the former referee, although there were, in their own opinion, more important lines of work which should be considered. Then, too, referees have taken up lines of work without consideration of methods already adopted by the association or of work already done and reported upon. Perhaps some of these unsatisfactory conditions have been due to a lack of proper cooperation by the officers of the association or to failure to publish promptly the reports and recommendations of referees, but they are for the most part, in my opinion, due to a faulty plan of operation. No method is provided whereby the referee or associate referee shall notify or even consult with any committee or officer of the association concerning his plans for work at the beginning of his term of appointment or of the progress he is making during the year. Often the first and only information that the Committee on Recommendations of Referees receives concerning the line of work a referee is engaged in, is the referee's report, which comes to hand a few days before the annual meeting, and failure to receive a report is often the first and only information the committee receives that a referee has not been active during the year. This unsatis-

factory condition is also revealed in the association methods and is forcibly brought to the attention of the Committee on Editing Methods of Analysis every time a revision of the *Book of Methods* is undertaken. Obsolete and unused methods have been left in the *Book of Methods* for the reason that the referees, intent upon their own special lines of work, recommend the adoption of a method without considering the disposition to be made of methods for the same determination already adopted by the association. These conditions, however, are but a natural sequence in the development of our association. Beginning as it did as a simple organization dealing with a single line of products in which every member was vitally interested, it has developed into a very complex organization, dealing with subjects so great in number and so different in kind that no one member can be intimately acquainted with all of them.

As this is our fortieth anniversary perhaps it may not be out of place for me to relate a little of the early history of our association, particularly as it bears upon this question of organization, which I desire to bring to your attention. As is well known to many of you, the association was officially organized at Philadelphia, Pa., on September 9, 1884, by a small group of chemists connected with the State Departments of Agriculture, the Agricultural Experiment Stations, and the U. S. Department of Agriculture and who were interested in the analysis of commercial fertilizers. According to the report of the proceedings of the Philadelphia meeting representatives from six States and the U. S. Department of Agriculture were present. The organization meeting at Philadelphia, however, was not the first meeting of the chemists interested in agricultural chemistry in this country. Four meetings of chemists and other officials interested in fertilizer control were held prior to that date, the first in Washington in 1880, in response to a call issued by the Hon. J. T. Henderson, Commissioner of Agriculture for the State of Georgia. This was followed by a meeting during the same year at Boston, Mass.; another in 1881 at Cincinnati, Ohio; and a fourth at Atlanta, Ga., in May, 1884. The report of the address of the presiding officer, the Hon. J. T. Henderson, at the meeting in Atlanta, gives us considerable information concerning the conditions that brought about these early conferences. He spoke as follows:

"Only four years ago I was not aware that there had been any effort made to secure the adoption of a uniform method and rules for the conduct of fertilizer analyses, although the importance of such a uniform system must have often occurred to those connected with the trade in fertilizers, as manufacturers, chemists, or boards of fertilizer control. Shortly after my accession to the office I now have the honor to fill, such a necessity occurred to me with very great force. When I sent the official report of my chemists to manufacturers, it was frequently met

with complaints that the results were too low. In some cases I had reason to believe that the analyses so reported were higher than manufacturers expected. I soon became satisfied of two things: First, that the work of the State chemist was entitled to the highest confidence, because verified and sustained by some of the most skillful and experienced chemists in the land, some of whom are now before me; second, that the difficulty was due solely to the use of radically different methods in the laboratory. I, thereupon, after consultation with many of you now present, called a convention of representative analytical chemists, official and private, to consider the question. That convention, as you all know, met at Washington, D. C., on July 26th, 1880. With its work you are all familiar. A provisional method, now universally known as the Washington Method, was adopted by the convention, and generally by the chemists of the country in their laboratories. Adjourning to Boston, the members present formed themselves into a section of the American Association for the Advancement of Science.

"At the meeting of the Association at Cincinnati on the 18th of August, 1881, the Chemical Section made some changes in the Washington Method, radical in character, with the provision that the method so adopted should be of force during the year. Since that meeting many valuable experiments have doubtless been made by you, and much additional experience gained in the use of methods, and it is to be hoped that you are now prepared to adopt a satisfactory and permanent system. In calling this convention thus early in the season and in advance of the annual meeting of the Association for the Advancement of Science, I have been actuated by a sense of the importance of adopting a uniform, and if possible, a more generally satisfactory method of analysis in time to enable manufacturers, if necessary, to conform to the same in promulgating their work of and for the next season."

At the Atlanta meeting a committee of three was appointed to consider plans and take steps looking to the permanent organization of this group of chemists. This committee reported at the meeting held in Philadelphia, Pa., the following September, recommending the formation of two organizations: (1) An Association of Official Chemists to be entirely distinct from the American Association for the Advancement of Science, to which should be left the decisions as to methods of analysis, etc., and (2) a sub-section of the American Association for the Advancement of Science, open to all agricultural chemists for purposes of investigation and discussion. Apparently the principal question at issue was whether a separate association should be formed or the group become a section of the American Association for the Advancement of Science. The report of the meeting states that "the discussion which followed developed an almost unanimous opinion that the objects of the convention would not be advanced by a union with the American Association,

but would be best subserved by the formation and maintenance of a separate organization". A committee of five was appointed to report on a form of organization, which committee reported the following day, September 9, 1884, submitting a constitution for a separate organization. The report was adopted and thus was officially created the organization which has done so much to further scientific agriculture in this and other countries.

It is interesting to note that the constitution adopted at the organization stated the object of the association as follows: "To secure, as far as possible, uniformity in legislation with regard to the regulation of the sale of commercial fertilizers in the different States and uniformity and accuracy in the methods and results of fertilizer analysis". If the matter of legislation was ever seriously considered apparently wise counsel prevailed for the reports of subsequent meetings show little activity along that line, the principal action appearing to have been the adoption in 1899 of the report of a special committee on uniform methods of fertilizer control giving a statement of principles to be followed in formulating State or national fertilizer legislation. The association early appreciated that its principal usefulness lay in the studies of methods of analysis, and this has never been lost sight of during the 40 years of its existence. At the meeting in Philadelphia three committees, corresponding somewhat to our present referee system, were appointed, one on phosphoric acid, one on nitrogen, and one on potash, and two methods, one for the determination of phosphoric acid and one for potash in commercial fertilizers, were adopted. The methods apparently were adopted for the season or year and were published as a part of the proceedings.

Although organized for the particular purpose of studying methods for the analysis of fertilizers it was but natural that the association would soon widen its activities. And so we find that as early as 1886 it was decided to include the study of methods for the analysis of cattle foods and dairy products. The following subjects were added in later years: In 1887, methods for sugars and fermented liquors; in 1890, methods for soils and ash; in 1894, methods for tanning materials and leather; in 1898, methods for insecticides; in 1901, methods for food adulteration; in 1903, methods for medicinal plants and drugs; and in 1908, methods for water. Last year we added agricultural liming materials and naval stores. Our constitution no longer limits the scope of activities to commercial fertilizers but gives as the object of the association "to secure uniformity and accuracy in the methods, results, and modes of statement of analysis of fertilizers, soils, cattle foods, dairy products, and other materials connected with agricultural industry". Instead of three committees of three members each studying the methods for the analysis of a single class of products, we



now have about 30 referees and 50 associate referees studying methods for more than 30 different classes of products; a committee of 9 members to consider the reports of these referees and associate referees; and 10 other committees composed of from 3 to 11 members each to consider general and special activities in which the association is engaged. This complex organization is necessary as a safeguard against precipitate action and is in keeping with the specialization tendencies of the age in which we live. Our need now is for some method of coordinating these numerous and varied activities to the end that our forces may be concentrated and utilized to the best interests of the association. During the past year your officers endeavored to devise and to put into operation such a method, which for want of a better name, we designated as the project plan of operation. The principal features of the plan are as follows: (1) All work on methods for a product or for a group of similar products, as usually represented by a chapter in the *Book of Methods*, shall be placed under the direction of a referee, designated as general referee, who shall be responsible for all the methods under his subject and each line of work under a general subject shall be placed in the hands of an associate referee, who advises with, and reports to, the general referee. (2) Each associate referee shall immediately after acceptance of appointment submit to the general referee an outline of the work he proposes to do during the year, this outline to be based upon the recommendations of the previous referee, as considered and reported upon by the Committee on Recommendations of Referees and approved by the association. (3) The general referee shall consider the plan submitted by the associate referee, particularly for proper coordination with the work of other associate referees on his subject, existing association methods, work previously done, etc., and shall notify the associate referee of his approval of the plan submitted or of any modifications deemed necessary. The general referee shall also submit to the Chairman of the Committee on Recommendations of Referees an outline of the final plans for work agreed upon for the associate referees of his subject. Similarly, referees operating independently shall notify the Chairman of the Committee on Recommendation of Referees of their plans of work for the year. (4) Each associate referee shall submit a brief report of the progress of his work at quarterly intervals to the general referee under whom he operates, and the general referees and independent referees shall in a similar manner make brief reports of the progress of the work on their respective subjects at quarterly intervals to the Chairman of the Committee on Recommendations of Referees.

This plan was explained to as many of the referees and associate referees as could be assembled at the last meeting of the association. Not all referees and associate referees have followed this procedure during

the past year. Probably some did not know about it, others did not understand the procedure, and still others did not appreciate the reason for a change. But a sufficient number did follow the plan to indicate that such a procedure will not only greatly assist in coordinating the activities of the association but will also greatly stimulate the work of the referees and associate referees. There is nothing particularly new in this method of procedure; it is simply an application to the work of the association of the principles of organization in the same manner as they are applied to the properly organized research departments of our colleges and universities and similar departments connected with private corporations. Its purpose is to determine carefully in advance the line of work of a referee or associate referee by consideration of all data and information concerning previous work done, existing methods, relative need for the proposed method, work of other referees, etc.; and once having determined upon a line of work to carry it through to completion, keeping the proper officials of the association advised at frequent intervals of the progress of the work.

There are two or three matters concerning the work of the association that I desire to bring to your attention. Perhaps at some future date some officer of the association will consider it worth while to pursue some of these further. The first is with reference to a standardization of the association methods. There are in the *Book of Methods* many instances where two or more methods are given for the same determination, and the number of these instances is rapidly increasing. If these duplicate and triplicate methods gave identical results—that is, if chemistry was the exact science many people believe it to be—perhaps it would not matter, although even then it would seem preferable that there should be one and only one designated standard method of the association for a given determination. But we know that in many instances there is a material difference in the results obtained by these duplicate methods, and even where the differences are small they are of considerable importance commercially and may be embarrassing to an analyst in a court action. Not only do we have instances of two or more methods for the same determination in one chapter, but we also have in the different chapters similar duplications of methods for determining the same element. For instance, in the determination of calcium the calcium oxalate precipitate is ignited and weighed as calcium oxide; mixed with ammonium sulfate and ignited and weighed as calcium sulfate; and dissolved in varying strengths of dilute sulfuric acid and titrated with standard potassium permanganate solution. Perhaps the most conspicuous example of these duplications is that of the six methods given for the determination of reduced copper in the reducing sugar methods under “Sugars and Sugar Products”, none of which is exactly the same as the methods for copper in the other chapters. Has not the time

arrived when our association should give some consideration to the question of standardization of our methods not only for each chapter but throughout the *Book of Methods*?

Similarly, throughout the *Book of Methods* there is a lack of uniformity in, or an entire absence of directions for, the form of statement of analytical results. One of the objects of the association, as stated in the first paragraph of the constitution, is "to secure uniformity \* \* \* in \* \* \* modes of statement of analysis". Apparently this has been lost sight of in our consideration and adoption of analytical methods. In the present revision of the *Book of Methods* your Committee on Editing Methods of Analysis has endeavored to supply these directions where missing and to standardize the form of expression used wherever possible. But this is not always possible without further consideration by a referee and the association. The form of statement of results should be included in every analytical method adopted by the association, but before adoption the form used in connection with the same determination in other products and in other chapters should be considered with a view to uniformity and to a standard form of statement in so far as this is possible.

When I started to prepare this paper I was much concerned as to whether or not the association matters I had in mind to bring to your attention would make a paper of reasonable length, but as I attempted to outline them I became much more concerned as to whether or not I would be able to include them all without making my address too long and tiresome. Just two or three more suggestions and I will have finished.

The officers of our association are continually having brought to their attention information to the effect that other associations are engaged in the development or in the perfection of methods of analysis that relate to the same products and that even duplicate those of this association. It is unfortunate to waste time and energy in duplicating work already done and even more unfortunate to have other associations adopt methods differing from an official method, but perhaps to a considerable extent we have ourselves to blame for this situation. I wonder, for instance, if we have kept ourselves fully informed as to what the needs of these other associations in the matter of methods for the analysis of agricultural products really are, and if we have tried in every way to cooperate with them and to supply their needs. I even wonder sometimes if we as an association know whether or not we are supplying our own members with tested and efficient methods for all the determinations in the varied lines of agricultural chemistry they are called upon to perform. We should have a definite arrangement whereby the need for new methods, the shortcomings of existing methods, and the duplication of studies of methods for the analysis of agricultural products by other associations will be brought to the attention of the secretary of our own

association. Upon receipt of such information he would proceed at once with the necessary steps to meet the deficiency and to cooperate to the fullest extent in the work of any association that has to do with methods for the analysis of agricultural products. If any of the official methods are inaccurate, we should be first to discover the inaccuracy. If better or more practical methods are available, we should have no hesitancy in making them available for use by our own members through proper study and action by the association.

The association has, in a few instances, considered methods of sampling; the outstanding instance was the report presented at the 1919 meeting of the association by the special committee on sampling of fertilizers appointed to cooperate with a similar committee of the American Chemical Society. The recommendations of that committee were adopted, and the association now has an official method for the sampling of commercial fertilizers. The methods for soils have always included directions for sampling, and now directions for sampling have been adopted by the association for butter, cheese, milk, and a few other products. The need for official methods of sampling practically all agricultural products and particularly those products subject to regulatory laws enforced by members of the association has long been appreciated. The magnitude of the undertaking has probably been the cause of our delayed action. Appreciating the necessity for accurate and practical methods of sampling in the enforcement of regulatory laws through many years of service as a regulatory official I recommended to the executive committee of the association the appointment of a committee to consider the matter in all its phases and, if possible, to submit at this meeting for your consideration a plan for the development and perfection of methods for sampling the various products covered by the official methods of analysis. The recommendation was approved by the executive committee. The special committee was appointed, and their report will be submitted for your consideration.

The suggestion has been made that the association should appoint referees and associate referees for the study of methods for the analysis of paints and paint ingredients, laws governing the manufacture and sale of which are enforced by official departments embraced in the membership of this association. The executive committee, being without full information concerning the need for such methods of analysis and the status of work done by other associations, decided to recommend the appointment of a committee to make these investigations and report at the present meeting. The committee will submit its recommendations for your consideration.

At the annual meeting last year, two or three open conferences on subjects of interest to small groups of members were held. This, in my opinion, should be encouraged. One of the greatest benefits derived

from attendance at these meetings is the opportunity afforded of meeting and discussing with others engaged in the same line of work problems of mutual interest. This is furthered by these group conferences. Our membership is not large enough nor would the best interests of the association warrant the creation of further sections or divisions, but there is no reason why members and visitors interested in the same or similar lines of chemical work should not arrange for informal group conferences of this kind. At our meeting last year a number of members were disappointed because they did not learn of the conferences until too late to attend. This year we have requested that arrangements for these meetings be made with the secretary of the association in order that a time may be fixed which will not interfere with the program of the regular meeting, and that announcement may be made, giving everyone who desires an opportunity to attend. In his presidential address two years ago Dr. Veitch emphasized the value and importance of having unlimited discussion of the important subjects presented at our annual conventions. Perhaps a few informal group conferences would help to solve the difficulty of providing sufficient time for the thorough discussion of some of the matters of much importance to small groups of members.

I appreciate that these remarks have been disconnected and that I have rambled over nearly every phase of the association's activities, including past history. However, this was necessary if I carried out what I conceive one of the duties of your presiding officer to be—that is, to bring to your attention those matters concerning the association's welfare that appear of greatest importance for its continued development along the well planned and conservative lines that have characterized its past.

If Dr. Wiley were not present, I would say that our association is rapidly passing into the hands of a third generation. Our fondest hope is that all future generations will be imbued with the same enthusiasm, steadfastness of purpose, and high ideals that characterized that small group of chemists that met in the city of Philadelphia 40 years ago last month and founded the institution which we so dearly love.

## ORDER OF PUBLICATION.

The order of publication adopted two years ago will be followed this year. The reports of the committees, presented on the last day of the annual meeting, will be given at the beginning of the proceedings rather than in their chronological order. This will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

### THIRD DAY.

### WEDNESDAY—MORNING SESSION.

#### REPORT OF THE REPRESENTATIVE AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The third annual meeting of the National Conference on Pharmaceutical Research was held at Buffalo, New York, August 23, 1924. The representative of the Association of Official Agricultural Chemists was present as an unofficial observer.

The National Conference on Pharmaceutical Research had its origin in resolutions passed by several pharmaceutical associations at their 1921-1922 meetings. As a result the Research Conference was formed at Cleveland, Ohio, in 1922, with representatives of seven organizations interested in pharmacy.

According to the present constitution, the object of the conference is to encourage and stimulate in every possible way pharmaceutical research, using that term in its broadest sense. The conference serves as a clearing house of American pharmaceutical research. It is also concerned with funds encouraging research, compiling lists of research workers and problems to be studied, devising plans and suggesting topics for research for graduate students at pharmacy schools, and co-ordinating the work of several pharmaceutical organizations for doing research work. At the Buffalo meeting delegates were present from the following ten organizations:

American Conference of Pharmaceutical Faculties;  
American Drug Manufacturers' Association;  
American Pharmaceutical Association;  
American Pharmaceutical Manufacturers' Association;  
Bureau of Chemistry, U. S. Dept. of Agriculture;

National Association of Boards of Pharmacy;  
National Association of Retail Druggists;  
National Formulary Revision Committee;  
Proprietary Association; and  
U. S. Pharmacopeia Revision Committee.

In addition, unofficial observers were present from the following organizations:

Council on Pharmacy and Chemistry, A. M. A.;  
Section on Medicinal Chemicals, A. C. S.;  
Plant Science Seminar Group;  
Association of Official Agricultural Chemists; and  
Joint Committee on Definitions and Standards of Foods and Drugs.

A committee on research topics submitted a list of about fifty problems connected with pharmacy which require investigation between the meetings of the conference. The work is carried on by ten standing committees on the following subjects:

Dispensing Pharmacy;  
Manufacture of U. S. P. and N. F. Galenicals;  
Standardization of U. S. P. and N. F. Galenicals;  
Manufacture of Medicinal Chemicals;  
Standardization of Medicinal Chemicals;  
Sources and Identification of Botanic Drugs;  
Standardization of Botanic Drugs;  
Chemistry of Drug Plants;  
Biological Products; and  
Business Research in Pharmacy.

The meetings of the conference have been held in connection with the annual meeting of the American Pharmaceutical Association. The constitution provides for annual dues from each constituent organization of \$20.00.

With regard to dues, it might be mentioned that at the last meeting a motion was adopted directing the admission into the conference, without fee, of scientific bureaus of the U. S. Government and of three other research organizations, namely:

The Plant Science Seminar;  
The Joint Committee on Definitions and Standards of Foods and Drugs; and  
The Pharmaceutical Laboratory Seminar.

The pharmaceutical associations are to be congratulated in having formed this conference for the purpose of encouraging research and coordinating work of a research nature.

As this association is primarily interested in methods of analysis, the only point of interest in common between this association and the conference would be methods of analysis of drug products, drug plants, etc. This constitutes but a small part of the work of the conference at

the present time. It is not believed that this association can materially assist the conference by becoming affiliated with it as a member of the organization, and it does not appear that the association would greatly benefit by such affiliation. It is not recommended, therefore, that this association apply for membership in the conference at this time.

It is thought, however, that if possible the association should have present at future meetings of the conference an unofficial observer to keep in touch with its work and to offer any information it might desire as to the activities of this association with respect to drug and pharmaceutical products. At some future time, when its work becomes more developed, it might appear advisable to affiliate definitely with the conference.

H. J. HUMPHREY.

Approved.

## REPORT OF COMMITTEE ON EDITING METHODS OF ANALYSIS.

The Committee on Editing Methods of Analysis has been very active during the past year. Immediately following the close of the 1923 meeting the members began the task of incorporating the additions and changes which had been made to the several chapters of methods since the last revision, preparatory to final editing for the printer. This work, with the necessary correspondence with referees, associate referees, authors, and others, has been so time-consuming that only recently have the chapters been completed for final editing by the associate editor of *The Journal*. The committee regrets this delay, but the task was so great that it could not be avoided if the work was to be done in the thorough manner characteristic of all undertakings of the association.

All the chapters of methods are now practically ready for the printer, and the manuscript for some of the chapters has been delivered to the printer. The remaining ones will follow rapidly, and unless further unforeseen delays occur, it is expected that the revised *Book of Methods* will be ready for distribution soon after January 1, 1925.

The committee desires to take this opportunity to express its appreciation to the referees, associate referees, and others for the valuable assistance given in the revision work and especially to the associate editor of *The Journal*, Miss Marian E. Lapp, who not only has attended to the final editing of all manuscript for the printer but throughout the work has cooperated with and assisted the committee in many ways.

Though the detailed work has at times been trying, the splendid spirit of cooperation and help shown by everyone who has been called upon to assist has made the revision work a delightful experience in many ways.



In connection with the correspondence on the methods, several suggestions concerning the form and arrangement of the *Book of Methods* have been received. These came too late for consideration in the revision just completed and are therefore here recorded for the information of future editing committees. The most important of these are as follows:

(1) That all reagents used, together with directions for preparation, be listed alphabetically and placed in a chapter, preferably in the front of the book, similar to the form followed in the United States Pharmacopeia.

(2) That all standard solutions, together with directions for preparation, be listed and placed in a separate chapter.

(3) That the apparatus used in the methods be listed and described in a separate chapter.

(4) That the commonly used standardized determinations, such as moisture, ash, nitrogen, ether extract, alcohol, etc., be grouped together and made the first chapter of the book.

(5) That the strength of all reagents be stated in the terms of normality.

(6) That the chapters be arranged alphabetically.

Inasmuch as the revised *Book of Methods* includes all additions and changes made at the 1923 meeting of the association, a recapitulation of these additions and changes is not included as a part of this report, as has heretofore been done.

R. E. DOOLITTLE, *Chairman*.

Approved.

## REPORT OF THE BOARD OF EDITORS.

By R. W. BALCOM (Bureau of Chemistry, Washington, D. C.), *Chairman*.

The year has been a fairly successful one for *The Journal*. The number of subscriptions on our books at this time is 810, as compared with 801 a year ago. A slight decrease in the number of domestic subscriptions has been more than offset by the increase in the number of foreign subscriptions, which now constitute  $16\frac{2}{3}$  per cent or one-sixth of the total. As a matter of policy, mainly for the purpose of making *The Journal* available to foreign abstracters, the Board of Editors has deemed it advisable to initiate exchanges to a limited extent. Exchange is now being made with seven foreign publications.

The cost to the association of the four numbers of *The Journal* issued between November 1 of last year and October 1 of this year was \$3,875.52. Receipts from *Journal* subscriptions and advertisements during the same period were \$4,250.53, the difference in favor of the association being about enough to defray office expenses, consisting principally of disbursements for wrappers, stationery, and postage. Total receipts, as shown

in the financial report on publications, includes an item of \$623.56 for *Methods of Analysis*, most of which was available for use in decreasing the still existing deficit on publications. This \$623.56 came from the sale of the few copies of the 1920 edition of *Methods of Analysis* remaining at the time of the last meeting, from collections on sales made before that date, and from advance payments, amounting to \$96.50, accompanying orders for the new edition about to be issued.

Relations with the printers have been very satisfactory throughout the year. Good service has been rendered by them, and bills have been paid without any undue delay. It is possible to report for the first time since the board was created that all bills now due have been received and paid.

The number of papers submitted for publication in the "Contributed Papers" section of *The Journal* during the year has been encouraging. To remove the distinct disadvantage under which the Board of Editors is now laboring in its efforts to strengthen this section, the association should provide fifty reprints without covers free of charge to the authors of contributed papers. At least as much as this is done by nearly all other scientific journals.

It has been suggested that the volume numbers of *The Journal* should be made to coincide with the calendar year so that the complete proceedings of any meeting of the association would be found in one volume, the four numbers of which would issue during the calendar year immediately following that meeting. This change is very desirable and should be put into effect as soon as possible.

This matter of reprints and of making the volume numbers of *The Journal* coincide with the calendar year is mentioned at this time so that the board may be guided by the opinion of the association if there is any question as to the desirability of putting these plans into effect as soon as it may appear practicable to do so.

A detailed financial statement of receipts and disbursements by the board from Nov. 1, 1923, to Oct. 1, 1924, is appended.

## FINANCIAL REPORT ON PUBLICATIONS FROM

By R. W. BALCOM (Bureau of Chemistry,

## RECEIPTS.

1923			
Oct. 31	Bank balance.....		\$606.73
	Total deposits.....	\$4,936.21	
	Less redeposited checks.....	31.00	
		<u>4,905.21</u>	
			<u>\$5,511.94</u>

## DETAILED STATEMENT RELATIVE TO RECEIPTS.

*Journal subscriptions.*

No. ordered	Price each	Total	
45	\$5.50	\$247.50	
453	5.00	2,265.00	
87	4.40	382.80	
228	4.00	912.00	
1	3.75	3.75	
1	3.36	3.36	
1	3.25	3.25	
2	2.80	5.60	
4	2.50	10.00	
2	2.00	4.00	
35	1.50	52.50	
4	1.25	5.00	
5	1.20	6.00	
5	1.00	5.00	
Total		<u>\$3,905.76</u>	
Less loss on exchange		.23	
Total			<u>\$3,905.53</u>

*Methods subscriptions.*

No. ordered	Price each	Total	
14*	\$5.50	\$77.00	
76†	5.00	380.00	
7	4.40	30.80	
33	4.00	132.00	
1	3.00‡	3.00	
Total		<u>\$622.80</u>	
Plus gain on exchange		.76	
Total			<u>623.56</u>

*Advertisements.*

No ordered	Price each	Total	
12	\$25.00	\$300.00	
3	15.00	45.00	
Total			<u>345.00</u>

*Reprints.*

University of Tennessee	9.07	
Total, Journal, Methods, Ads, Reprints		4,883.16
Plus bank balance		606.73
Plus checks returned because of excess payment		22.05
Total		<u>\$5,511.94</u>

\* Includes 3 orders for new edition upon which payment was made in advance.

† Includes 16 orders for new edition upon which payment was made in advance.

‡ Damaged copy.

NOVEMBER 1, 1923, TO OCTOBER 1, 1924.

Washington, D. C.), *Chairman, Board of Editors.*

## DISBURSEMENTS.

		Amount	Check No.
1923			
Nov. 16	Janet K. Smith, office expenses . . . . .	\$25.00	70
Dec. 8	Industrial Printing Co., payment of bill of 8/31/23 . . . . .	963.65	71
Dec. 13	L. B. Burnett, payment for back numbers of <i>Journal</i> . . . . .	4.31	72
Dec. 14	Harry J. Bastone, payment for back numbers of <i>Journal</i> . . . . .	3.75	73
Dec. 22	Postmaster, Washington, D. C., box rent for quarter ending 3/31/24 . . . . .	2.00	74
Dec. 28	Janet K. Smith, office expenses . . . . .	25.00	75
1924			
Jan. 7	Postmaster, Washington, D. C., 5000 special request window envelopes . . . . .	119.30	76
Jan. 21	Industrial Printing Co., payment of bill of 11/27/23 . . . . .	635.25	77
Jan. 24	R. P. Andrews Paper Co., payment of bill of 1/22/24 . . . . .	6.25	78
Apr. 3	Janet K. Smith, office expenses . . . . .	25.00	79
Apr. 4	Moore-Cottrell Subscription Agency, reimbursement for over- payment on <i>Journal</i> subscriptions . . . . .	8.55	80
Apr. 4	A. L. Hollister, reimbursement for overpayment on <i>Book of Methods</i> . . . . .	2.00	81
Apr. 17	Williams and Wilkins, six copies of Vol. III, No. 1 . . . . .	7.50	82
Apr. 17	Industrial Printing Co., payment of bills of 2/11/24 and 4/11/24 . . . . .	33.07	83
Apr. 17	Industrial Printing Co., payment on account of bill of 3/31/24 . . . . .	600.00	84
May 5	Shirley Laboratories, reimbursement for Vol. VII, No. 3 . . . . .	1.50	85
June 17	Postmaster, Washington, D. C., box rent for quarter ending 9/30/24 . . . . .	2.00	86
July 12	Industrial Printing Co., payment balance of bill of 3/31/24 . . . . .	610.65	87
July 15	Postmaster, Washington, D. C., for mailing <i>Journals</i> . . . . .	15.00	88
July 16	Janet K. Smith, office expenses . . . . .	25.00	89
July 26	Industrial Printing Co., on account of bill of 5/31/24 . . . . .	600.00	90
Aug. 11	Industrial Printing Co., payment balance of bill of 5/31/24 . . . . .	653.44	91
Aug. 14	McKesson and Robbins, reimbursement for duplicate pay- ment of <i>Journal</i> . . . . .	5.00	92
Aug. 20	Jose Santos, reimbursement for <i>Book of Methods</i> . . . . .	5.00	93
Aug. 22	Leicester Patton, payment for back numbers of <i>Journal</i> . . . . .	6.00	94
Aug. 29	Industrial Printing Co., payment of bill of 8/28/24 . . . . .	40.85	95
Sept. 19	Janet K. Smith, reimbursement for payment of bill of Co- lonial Printing Co., of 9/2/24 . . . . .	5.00	96
Sept. 23	Industrial Printing Co., payment of bills of 8/30/24 . . . . .	776.37	97
Sept. 23	Postmaster, Washington, D. C., box rent for quarter ending 12/31/24 . . . . .	2.00	98
Sept. 29	Janet K. Smith, office expenses . . . . .	25.00	99
	Bank balance, Sept. 30 . . . . .	\$280.50	
	Less outstanding check No. 98 . . . . .	2.00	
		<hr/> 278.50	

FINANCIAL REPORT OF THE SECRETARY-TREASURER

By W. W. SKINNER (Bureau of

RECEIPTS.

1923		
Nov. 1	Bank balance.....	\$414.54
	Dues for 1923 received too late for inclusion in 1923 report,	
	5 at \$5.00.....	\$25.00
	Dues for 1924 from institutional members, 69 at \$5.00.....	345.00
		<hr/>
		370.00

Total..... \$784.54

FROM NOVEMBER 1, 1923, TO OCTOBER 1, 1924.

Chemistry, Washington, D. C.).

# DISBURSEMENTS.

		Amount	Check No.
1923			
Nov. 16	Janet K. Smith, reimbursement for expenses 1923 meet- ing . . . . .	\$25.00	31
1924			
Feb. 16	Janet K. Smith, reimbursement for postage, etc., advanced from <i>Journal</i> office expenses . . . . .	10.00	32
Mar. 21	Postmaster, Washington, D. C., box rent for period 4/1/24 to 6/30/24 . . . . .	2.00	33
Aug. 18	Janet K. Smith, postage for mailing announcements of 1924 meeting . . . . .	25.00	34
Sept. 2	Industrial Printing Co., payment of bill of 8/29/24 . .	37.00	35
Sept. 30	Bank balance . . . . .	685.54	

Total . . . . . \$784.54

No report was made by the Committee on Quartz-Plate Standardization and Normal Weight.

## REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS.

At the open meeting of the Committee on Definitions of Terms and Interpretation of Results on Fertilizers G. S. Fraps presided in the absence of H. D. Haskins, chairman of the committee. Lively interest was shown in the discussion and in the presentation of the new definitions formulated by the committee.

The committee recommended the following definitions and interpretations of terms:

### *Second Reading as Tentative Definitions.*

#### 1. BASIC PHOSPHATE SLAG.

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and should contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ( $P_2O_5$ ), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid solution according to the Wagner method of analysis. Any other phosphate slag not conforming to this definition shall be designated *low grade*.

#### 2. INTERPRETATION OF THE WORD "LIME" AS APPLIED TO FERTILIZERS.

The term *lime* shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials, unless the lime is in a form to neutralize soil acidity, such as the oxide, hydroxide, or carbonate, or equivalent magnesia compounds.

#### 3. DRIED PULVERIZED OR SHREDDED MANURES.

*Dried pulverized or shredded manures* shall be only what the name indicates, and not mixtures of manures and other materials.

#### 4. MANURE SALTS.

*Manure salts* shall be understood to mean potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ( $K_2O$ ). The term *double manure salts* should be discontinued.

#### 5. SULFATE OF POTASH-MAGNESIA.

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ( $K_2O$ ), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and five-tenths per cent (2.5%) of chlorine.

#### 6. INTERPRETATION OF RESULTS ON ORGANIC NITROGEN IN MIXED FERTILIZERS.

On account of changes that have been made by the committee in the interpretations regarding this subject they will appear in the present report under the heading "First Reading as Tentative".

*First Reading as Tentative.***1. INTERPRETATION OF BRAND NAME.**

The *brand name* should include the grade of fertilizer, depending upon the section of country in which the product is sold. For example: 4-8-4 grade is interpreted in the northern section of the country as 4 per cent of nitrogen, 8 per cent of available phosphoric acid, and 4 per cent of potash; and in other sections, particularly in the southern section, the same grade is expressed as 8-4-4, referring to 8 per cent of available phosphoric acid, 4 per cent of nitrogen, and 4 per cent of potash.

**2. FERTILIZER FORMULA.**

The term *fertilizer formula* shall be interpreted as expressing the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16 per cent acid phosphate, 800 pounds of 9-20 tankage, and 400 pounds of sulfate of potash-magnesia.

**3. FERTILIZER GRADE.**

The term *fertilizer grade* as applied to mixed fertilizers shall designate the approximate proportion of nitrogen, available phosphoric acid, and water-soluble potash present in the mixture, and may be expressed in either of the following orders, according to the locality and custom: 4-8-6, meaning 4 per cent of nitrogen, 8 per cent of available phosphoric acid, and 6 per cent of potash; or 8-4-6, meaning 8 per cent of available phosphoric acid, 4 per cent of nitrogen, and 6 per cent of potash.

**4. ANALYSIS.**

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in terms of nitrogen, phosphoric acid, and potash in their various forms.

**5. BRAND AND BRAND NAME.**

A *brand* is a term, design, or trade mark used in connection with one or several grades of fertilizers.

A *brand name* is a specific designation applied to an individual fertilizer.

**6. UNIT.**

A *unit* of plant food is twenty pounds or 1 per cent of a ton of fertilizer.

**7. UNLEACHED WOOD ASHES.**

*Unleached wood ashes* are defined as ashes resulting from burning unleached wood and that have not had any part of their plant food extracted by contact with water or other solvent.

**8. LEACHED WOOD ASHES.**

*Leached wood ashes* are defined as ashes resulting from burning unleached wood, but as having had part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent.

**9. ASHES FROM LEACHED WOOD.**

*Ashes from leached wood* are defined as unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvents, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed.



## 10. DISSOLVED BONE.

*Dissolved bone* is defined as a ground bone or bone meal that has been treated with sulfuric acid.

## 11. FORM OF NITROGEN IN CYANAMID.

The nitrogen in calcium cyanamid shall be considered as being of organic nature.

## 12. ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS.

The following procedure is recommended in the study of the activity of the water-insoluble organic nitrogen in mixed fertilizers by means of the alkaline and neutral permanganate methods. These methods distinguish between good and poor sources of water-insoluble nitrogen and do not show the percentage availability of the material.

- (a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to 3/10 of 1 per cent (0.3%) or more of the weight of the material.
- (b) The water-insoluble nitrogen in mixed fertilizers showing activity below fifty per cent (50%) by the alkaline method and eighty per cent (80%) by the neutral method shall be classed as inferior; water-insoluble nitrogen activity fifty per cent (50%) or above by the alkaline method and eighty per cent (80%) or above by the neutral method shall be passed without adverse criticism.

The following topics are proposed for further consideration:

1. Statement of the meaning of the term "finely ground" in the definition of basic phosphate slag by means of the mesh sieve it should pass.
2. Allowance to be made for over-run of nitrogen, if any, in fertilizers of low activity.
3. Uniform methods of reporting results.
4. Maximum amounts of chlorine permissible in sulfate of potash and fertilizers in which the potash is claimed as sulfate.
5. Use of the terms "blood" and "bone" in connection with fertilizers not containing all their phosphoric acid and nitrogen in these forms.
6. Uniform order and terms in expressing the grade of fertilizers.

H. D. HASKINS,

E. G. PROULX,

R. N. BRACKETT,

J. W. KELLOGG.

G. S. FRAPS,

*Committee on Definitions of Terms and Interpretation of Results on Fertilizers.*

Approved.

## REPORT OF COMMITTEE ON REVISION OF METHODS FOR THE ANALYSIS OF SOILS.

This committee offered the results of its labors to the last meeting of the association, and as a consequence the methods that are being incorporated in the revised *Book of Methods* have been materially improved. The chairman has given considerable time to a collaborative effort with the chairman of the General Revision Committee in seeing that the methods for soils are in keeping and harmony with the methods in the

other chapters of the *Book of Methods*. The remaining members of the committee have materially assisted in this work through correspondence.

It has occurred to the chairman of this committee that it would be well to announce that criticism, both constructive and destructive, is welcomed. It is quite possible that a footnote in the proceedings, and possibly even in the *Book of Methods*, might attract the attention of those interested in methods for soil analysis. Unless such criticism is received, it devolves entirely upon the committee membership to ferret out any shortcomings that the methods may have.

W. H. MACINTIRE,	J. A. BIZZELL,
A. W. BLAIR,	A. G. MCCALL.
R. STEWART,	

*Committee on the Revision of Methods for the  
Analysis of Soils.*

Approved.

## REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES.

At the meeting of the association last year, the Committee on Recommendations of Referees presented a plan of operation designed to co-ordinate the work of the referees and associate referees. This plan, in brief, provided for the following: a more careful consideration of the recommendations made by referees and associate referees for work, the reporting at quarterly intervals of the progress made by referees and associate referees on subjects assigned, the continuation of a line of work agreed upon to a final conclusion, and a responsible head for each group or chapter of methods. This was quite fully explained in a letter sent to each referee and associate referee with the notice of appointment. This procedure, however, has not been followed by all referees and associate referees during the past year, but it has been complied with in every respect by a few and in part by many with the result that the Committee on Recommendations of Referees has been more fully advised concerning plans for work and more closely in touch with the progress being made by the referees and associate referees during the past year than in any former year. It is believed that the plan, if followed by all referees and associate referees, will not only accomplish the purpose intended but will greatly stimulate the collaborative work of the association.

The greatest difficulty at present appears to be the failure of referees to submit their reports at a sufficiently early date for Sub-committees A, B, and C to give them proper consideration. The association has endeavored for a number of years to require that these reports be sub-

mitted to the Chairman of the Committee on Recommendations of Referees at least thirty days before the annual meeting, in order that the individual members of the sub-committees may have time to study the reports and to take up with the referees by correspondence any questions of importance. But this has not been successful. Over 50 per cent of the referees' and associate referees' reports were received this year during the week immediately preceding the meeting. While this committee does not believe that it is advisable to change the date for submitting referees' reports, it does believe that it would be better for the members of Sub-committees A, B, and C to meet in Washington one or two days in advance of the annual meeting, to consider all referee and associate referee reports. It is thought that the work of the entire personnel of the committee at that time will accomplish a great deal more than can be accomplished in a much longer time by correspondence.

Another feature of handling the reports of referees and associate referees that is far from satisfactory is the lack of opportunity for members of the association to consider the recommendations made. Soon after the close of the 1923 meeting, the retiring president, A. J. Patten, suggested that this could be overcome if each referee would have mimeographed copies of an abstract of his report, including the recommendations, made and placed on the secretary's desk or other convenient place where those interested could secure a copy. This would mean that each referee—and there are about thirty—would prepare an abstract or summary of his report, including the reports and recommendations of the associate referees under him, have this mimeographed, and supply the secretary with fifty copies.

It is also the opinion of this committee that better consideration of the recommendations of referees and associate referees by the association would be secured if the Chairmen of Sub-committees A, B, and C submitted their reports at stated times to be given on the program instead of immediately following the reading of the referees' reports, as at present. It is practically impossible for these committees to have their reports ready in all cases when the referee concludes, particularly the first day of the meeting, and as a consequence members are often absent when the particular report in which they are interested is before the association for action. It is believed that this could be overcome, at least to a considerable extent, by having the report of each of these three sub-committees made a stated order of business on the regular program. This committee respectfully submits these three suggestions for the consideration of the association, believing that if put into effect some of the difficulties now existing will be overcome.

The committee wishes to express to the referees and associate referees its sincere appreciation of the splendid cooperation given during the past year in putting the new procedure into effect and to compliment these

officers of the association on the excellent reports made. The quality of the work covered by these reports will be more fully appreciated when the members of the association have an opportunity to consider the printed proceedings of the meeting.

R. E. DOOLITTLE, *Chairman*.

Approved.

## REPORT OF SUB-COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By W. H. MACINTIRE (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Chairman*.

[Waters, brine, and salt; tanning materials and leather; insecticides and fungicides; soils (liming materials, reaction values of soils); feeding stuffs (crude fiber, starch in the presence of interfering polysaccharides, stock feed adulteration); saccharine products (polariscopic methods, honey, maple products, maltose products, sugar house products, chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, potash); plants (sulfur and phosphorus in seeds of plants).]

### WATERS, BRINE, AND SALT.

It is recommended—

(1) That the method<sup>1</sup> for the determination of hydrogen sulfide in mineral waters be dropped. (First action changing an official method.)

Approved.

(2) That the method for the determination of hydrogen sulfide as described in the report of the referee be adopted as an official method. (First action as an official method.)

Approved with the qualification that collaboration be obtained before the second recommendation.

### TANNING MATERIALS.

No report or recommendations.

### INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the study of methods of analysis of emulsions be continued.

Approved.

(2) That the xylene distillation method for the determination of water, as described in the report of the referee, be studied as a method for the determination of water in soaps.

Approved.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 27.

(3) That the Kissling method<sup>1</sup> for the determination of nicotine in tobacco and tobacco extracts be dropped as an official method. (First action on an official method.)

Approved.

#### SOILS.

Concurrent recommendations with the associate referees.

Approved.

#### AGRICULTURAL LIMING MATERIALS.

It is recommended—

(1) That the modified Proctor method<sup>2</sup> for the determination of calcium oxide in burnt and hydrated limes be dropped as a tentative method.

Approved.

(2) That the modified Scaife method<sup>2</sup> and the Stone and Schuech method<sup>2</sup> for the determination of calcium oxide in burnt and hydrated limes, as modified by the associate referee, be further studied, particularly as to the influences exerted by impurities in the commercial product.

Approved.

#### REACTION VALUES OF SOILS.

It is recommended that the title of the associate referee be changed from "Associate Referee on Acidity Values of Soils" to "Associate Referee on Reaction Values of Soils".

Approved.

#### FEEDING STUFFS.

It is recommended that the method for the determination of moisture by distilling with toluene, as described in the paper presented by Messrs. Bidwell and Sterling (p. 295), be studied with the view to its adoption as an official method.

Approved.

#### CRUDE FIBER.

It is recommended that the method for the determination of crude fiber<sup>3</sup>, which was presented for adoption as official last year, be made an official method. (Final action.)

Approved.

#### STARCH IN THE PRESENCE OF INTERFERING POLYSACCHARIDES.

It is recommended—

(1) That the changes in the method for the determination of starch in the presence of interfering polysaccharides, submitted in the report of the associate referee, be incorporated in the method.

Approved.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 65

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7·254.

<sup>3</sup> *Ibid.*, 339.

(2) That the method as modified be adopted as official. (First action and subject to editorial revision and direction that collaborative study be carried out prior to offering the method for passage on second reading.)

Approved.

STOCK FEED ADULTERATION.

No report and no recommendations.

SUGARS AND SUGAR PRODUCTS.

It is recommended—

(1) That Section 4, Chapter VIII, "*Drying Upon Quartz Sand—Official*"<sup>1</sup>, be changed to provide for drying in vacuum at 70°C. in the case of products containing levulose, and that it be changed in certain details of technique. This section will then read as follows:

Digest pure quartz sand that will pass a 40-mesh sieve but not a 60-mesh with strong hydrochloric acid, wash free from acid, dry, and ignite. Preserve in a stoppered bottle.

Place 25–30 grams of the prepared sand and a short stirring rod in a flat-bottomed dish, approximately 60 mm. in diameter; dry thoroughly; cool in a desiccator; and weigh. Then add sufficient of the diluted sample to yield approximately 1 gram of dry matter and mix thoroughly with the sand. Heat on a steam bath for 15–20 minutes, stirring at intervals of 2–3 minutes or until the mass becomes too stiff to manipulate readily. Dry at 70°C. under a pressure of not to exceed 100 mm. of mercury, making trial weighings at 2 hour intervals until the change in weight does not exceed 2 mg. For substances containing no levulose or other readily decomposable matter, the determination may be made by drying at atmospheric pressure in a water oven at the temperature of boiling water, heating for 8–10 hours, cooling in a desiccator, weighing, and repeating the heating and weighing until the loss in 1 hour does not exceed 2 mg. Report the percentage loss in weight as moisture.

Approved.

(2) That Section 9, Chapter VIII, "*Refractometer Method—Official*", be changed so as to provide for a standard temperature of 20°C. instead of 28°C., with the substitution of the corresponding Schönrock tables for those of Geerligs'. The first part of this section will then read as follows:

Determine the refractometer reading of the solution at 20°C. and obtain the corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from Schönrock's table<sup>2</sup>, if the instrument gives readings in terms of refractive index. If the refractometer reading is obtained at a temperature other than 20°C., correct the result according to Stanek's correction table<sup>3</sup>.

Approved.

(3) That Section 12, Chapter VIII, "*Ash—Method III—Official*", be changed in title to "Sulfated Ash", and that results be reported, without correction, as percentage of sulfated ash. This section will then read as follows:

<sup>1</sup> The references in these recommendations will be found in *Assoc. Official Agr. Chemists, Methods*, 1920, VII and VIII.

<sup>2</sup> Bureau of Standards Circular 44, 134

<sup>3</sup> *Ibid.*, p. 137.

Weigh 5 grams of the sample into a 50–100 cc. platinum dish, add 0.5 cc. of concentrated sulfuric acid, ignite gently until the sample is well carbonized, and then burn in a muffle at low redness to constant weight. Express the result as percentage of sulfated ash.

Approved.

(4) That the recommendation of the Associate Referee on Polariscopic Methods relative to revision of Section 14, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Hydrochloric Acid—Official", be adopted.

Approved.

(5) That Section 15, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase—Official—Reagent", be revised so as to conform with the most approved method for preparation of invertase. This section will then read as follows:

*Invertase solution.*—Commercial invertase preparations are available on the market. If it is desired to prepare the solution in the laboratory, the following procedure may be used. In either case the preparation may be further purified and concentrated by the ultrafiltration method described below. Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temperature not to exceed 40°C.

*Preparation of crude invertase solution.*—Mix yeast with water in the proportion of 10 pounds of compressed baker's yeast with 5 liters of water. Add 2 liters of toluene and stir thoroughly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity through large fluted papers. Mix the residue with 2 liters of water, filter, and combine the filtrates. Purify by adding 15 grams of neutral lead acetate to each liter of extract and filtering on paper after all lead acetate has been dissolved. Complete purification immediately by dialysis or by washing on the ultrafilter described below.

*Preparation of a collodion ultrafilter.*—Dissolve 6 grams of Cooper's negative cotton (snowy) in a mixture of 50 cc. of absolute alcohol and 50 cc. of absolute ether. Add the alcohol to the cotton, allow it to stand in a stoppered flask 10 minutes, add the ether, and shake. Allow to stand overnight before using. Pour about 100 cc. into a 2000 cc. cylinder and coat the entire inside surface with the collodion. Drain and dry for 10 minutes. Fill with water, let stand 10–15 minutes, pour out the water, and remove the sack. Test for leaks by filling with water. Slit open longitudinally and cut out a circular piece about 7–8 inches in diameter. Cut the bottom from a 2 liter bottle or Erlenmeyer flask and grind the edge smooth. Place it upon the still moist collodion disc, fold the edge of the latter up around the bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place 3 or 4 thicknesses of wet filter paper in an 8 inch Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to the depth of an inch, between the bottle and inside of the funnel. Provide the bottle with a small mechanical stirring device.

*Washing and concentration of invertase solution by ultrafiltration.*—Filter 4 liters of the partially purified solution through the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled water introduced by means of a constant level device until the filtrate is colorless, 3 or 4 liters of wash water being required. During the entire process the invertase solution must be preserved with toluene or chinosol.

*Determining the activity of the invertase solution.*—It is generally sufficient to test the activity of the invertase solution as follows: Dilute 1 cc. of the invertase preparation to 200 cc. Transfer 10 grams of sucrose (granulated sugar) to a sugar flask graduated at 100 cc. and 110 cc., dissolve in about 75 cc. of water, add 2 drops of glacial acetic acid, and dilute to the 100 cc. mark. To the 100 cc. of sugar solution add 10 cc. of the dilute invertase solution and mix thoroughly and rapidly, noting the exact time at which the solutions are mixed. At the termination of exactly 60 minutes make a portion of the solution just distinctly alkaline to litmus with anhydrous sodium carbonate and determine the polarization in a 200 mm. tube at 20°C. If the invertase solution is sufficiently active, the alkaline solution will polarize approximately 30° Ventzke without correcting for the dilution to 110 cc. and the optical activity of the invertase solution.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 cc. of the invertase solution to 200 cc. at 20°C., place in a constant temperature bath at 20°C., and when the solution has attained the latter temperature pipet 20 cc. of it into a flask containing 200 cc. of a sucrose solution of 10 grams per 100 cc. concentration, which has been previously made distinctly acid to litmus paper by the addition of strong acetic acid and also brought to a temperature of 20°C. in the same bath. Mix thoroughly and promptly and note the time at which the invertase solution was added. Keep the sucrose-invertase mixture in the constant temperature bath; remove portions at the end of 15, 30, and 45 minutes; render each portion just distinctly alkaline to litmus paper with anhydrous sodium carbonate immediately after removing, and determine the polarization at 20°C. Correct all polarizations for the polarization of the invertase solution. Calculate the velocity constant,  $k$ , for each of the polarizations (at the times  $t$ ) subsequent to the initial polarization by the following formula

$$k = \frac{\log_{10} 1.32 R_0 - \log_{10} (R_t + 0.32 R_0)}{t}, \text{ in which}$$

$k$  = the unimolecular reaction velocity constant.

$t$  = number of minutes elapsing from time invertase and sucrose solutions were mixed until inversion was stopped by addition of sodium carbonate;

$R_0$  = initial polarization calculated by multiplying the polarization of the sucrose solution by 10/11 and correcting for the polarization of the invertase solution; and

$R_t$  = polarization at time  $t$ .

An invertase solution of sufficient activity should yield an average value for  $k$  (for the various periods) of at least 0.1 after multiplying the  $k$  value directly obtained by 200, in order to correct for the initial dilution of the invertase solution. The dilution of the invertase solution above mentioned is made solely for the purpose of determining its activity; the original, undiluted invertase solution is used as the inverting reagent in the determination of sucrose. The activity of the invertase preparation required for rapid inversion is the same as that needed for overnight inversion, but the proportion of invertase preparation used in the former case is twice that used in the latter instance.

An invertase preparation of the activity specified may be obtained from the Wallerstein Laboratories, 171 Madison Ave., New York City.

Approved.

(6) That the recommendation of the Associate Referee on Polariscopic Methods regarding revision of Section 16, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase—Official, be adopted.



(7) That the following paragraph providing for the rapid inversion with invertase solution be added to the "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase.—Official".

If a more rapid inversion is desired proceed as follows: Prepare the same sample as directed above and to 50 cc. of the lead-free filtrate in a 100 cc. volumetric flask add glacial acetic acid in sufficient quantity to render the solution distinctly acid to methyl red (used as outside indicator). The quantity of acetic acid required should be determined before pipetting the 50 cc. portion. Then add 10 cc. of invertase solution, mix thoroughly, place the flask in a water bath at 55°–60°C., and allow to stand at that temperature for 15 minutes with occasional shaking. Cool, add sodium carbonate solution until distinctly alkaline to litmus paper, dilute to 100 cc. at 20°C., mix well, and determine the polarization at 20°C. in a 200 mm. tube. Allow the solution to remain in the tube for 10 minutes and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. (If the solution has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposition of fructose ordinarily is more rapid than that of the other sugars present. If the solution has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase.) As further experience is acquired, the second polarization may be omitted when the analyst has satisfied himself that he is adding sodium carbonate in sufficient amount to complete mutarotation practically instantaneously without causing any destruction of sugar during the period intervening before polarization. Carefully note the reading and the temperature of the solution. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the formula given.

Approved.

(8) That the following formulas be substituted for the corresponding formulas now included in Section 17, Chapter VII, "Determination of Sucrose and Raffinose—Official", these changes being based upon revision of the Clerget divisor and raffinose inversion constant as published by Browne<sup>1</sup>:

(a) When the polarizations are made at 20°C.—

$$S = \frac{0.514 P - I}{0.844};$$

$$\text{whence } R = \frac{0.33 P + I}{1.563}.$$

(b) When the polarizations are made at temperatures other than 20°C.—

$$S = \frac{P (0.478 + 0.0018t) - I (1.006 - 0.0003T)}{(0.908 - 0.0032t) (1.006 - 0.0003T)},$$

$$R = \frac{P (0.43 - 0.005t) + I (1.006 - 0.0003T)}{(1.681 - 0.0059t) (1.006 - 0.0003T)}.$$

Approved.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1921, 13, 793.

(9) That in Section 20, Chapter VIII, "*Method II (Double dilution method)—Official*", the portion reading "The true direct polarization of the sample is the product of the two direct readings divided by their difference" be changed to read as follows:

The true direct polarization of the sample is equal to four times the direct polarization of the diluted solution less the direct polarization of the undiluted solution.

The true invert polarization is equal to four times the invert polarization of the diluted solution less the invert polarization of the undiluted solution.

Approved.

(10) That in Sections 21 and 22, Chapter VIII, "Commercial Glucose", *Methods I and II*, results be reported in terms of glucose solids, the factor 211 being used instead of 175 and 196 instead of 163. The factor 196 is a rounded figure based upon the average value 195.8 found by Bryan<sup>1</sup> and 196.2 recently found by Lathrop<sup>2</sup>. This change will eliminate variations due to differences in water content of the glucose.

Approved.

(11) That the subdivision "Maltose Products" under "Sugars and Sugar Products" be changed to "Starch Conversion Products", thereby including materials such as commercial glucose, dextrose, dextrins, etc.

Approved.

(12) That the work on sugars and sugar products during the next year be conducted along the lines recommended by the various associate referees.

Approved.

#### POLARISCOPIC METHODS.

It is recommended—

(1) That the text of the method for the determination of sucrose by inversion with hydrochloric acid be amended as given in the report of the associate referee. (First action amending an official method.)

Approved.

(2) That the text of the method for the determination of sucrose by inversion with invertase be amended as given in the report of the associate referee. (First action amending an official method.)

Approved.

(3) That further study be made of the analysis of sugar mixtures, and especially of mixtures containing sucrose and invert sugar, also amino acids and other impurities, as well as raffinose. The study should include not only the present methods of the association, but also those recommended by Jackson and Gillis<sup>3</sup> and others; special attention should be given to inversion at higher temperatures.

Approved.

<sup>1</sup> *J. Franklin Inst.*, 1911, 172: 337.

<sup>2</sup> Unpublished.

<sup>3</sup> U. S. Bur. Standard Sci. Paper 375, p. 153.

## HONEY.

It is recommended—

(1) That the following statement be inserted in the *Book of Methods*, VII, 45: "The resorcin test may be applied to all types of honey, but the aniline chloride test is of no value in the case of dark colored honey. (A positive test consists of a cherry-red color appearing at once; the yellow to salmon shades have no significance.)

Approved.

(2) That the resorcin test (Bryan's modification of Fiehe's test) and the aniline chloride test (Feder's), when positive, be considered conclusive evidence of the presence of commercial invert sugar in honey, provided the honey has not been stored for some length of time after having been heated to temperatures of upward of 160°F. (71.7°C.).

Approved.

(3) That the resorcin test and the aniline chloride test when negative be not regarded as conclusive evidence of the absence of commercial invert sugar sirup in honey.

Approved.

(4) That the studies of the resorcin and aniline chloride tests for honeys that have been stored for various periods of time after having been heated to temperatures that would prevail in the ordinary commercial handling of the product be continued.

Approved.

(5) That a study be made of the possibility of using the procedure of Auerbach and Bodländer<sup>1</sup> in the detection of adulteration of honey with invert sugar sirup.

Approved.

## MAPLE PRODUCTS.

It is recommended that a study of the Canadian method for the determination of the lead number of sirup be continued parallel with the Winton method as to accuracy obtainable and time required.

Approved.

## MALTOSE PRODUCTS.

It is recommended that the studies for the development of a reliable method for the determination of maltose be continued.

Approved.

## SUGAR HOUSE PRODUCTS.

It is recommended—

(1) That low-grade sugar be included in the collaborative studies of the coming year. This recommendation was amended by the committee

<sup>1</sup> *Z. Nahr. Genussm.*, 1924, 47: 233.

to include a study of the method of determining moisture by distillation with toluene as described in a paper presented by Bidwell and Sterling.

Approved.

(2) That the recommendations of the preceding year<sup>1</sup> relative to the method "Drying upon Pumice Stone—Official" and the proportions of sand in the method "Drying upon Quartz Sand—Official" be approved. (Final action.)

Approved.

(3) That efforts be made in the collaborative work of the coming year to diminish the number of methods for trial.

Approved.

(4) That collaborative work on the determination of moisture be continued.

Approved.

#### CHEMICAL METHODS FOR REDUCING SUGARS.

It is recommended that the following subjects be studied:

(1) The volumetric method in which methylene blue is employed as an inside indicator.

(2) The volumetric method in which the reduced copper or residual unreduced copper is determined iodometrically.

(3) The method of Quisumbing and Thomas<sup>2</sup>, or an equivalent method of high precision.

(4) A micro-chemical method.

(5) The iodine method for aldose sugars.

(6) The electrolytic method for copper.

(7) The reduction of copper in methyl alcohol vapor.

(8) A selective comparison of the methods now given in practical duplication.

(9) The limits of accuracy in the respective methods.

(10) A verification of the standard reference tables.

Approved.

#### FERTILIZERS.

It is recommended—

(1) That the following changes<sup>3</sup> be made in paragraph 5, "Preparation of Solution".

(1) That methods (a), (d), and (f) be deleted and the necessary changes in designation be made in lettering.

(2) That the statement "or 250 cc. if a 2.5 gram sample was used" be deleted.

(3) That to (a) now (b) add "Suitable for organic material like cottonseed meal alone or in mixtures".

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 264.

<sup>2</sup> *J. Am. Chem. Soc.*, 1921, 43: 1503.

<sup>3</sup> The references in these recommendations are to the *Assoc. Official Agr. Chemists, Methods*, 1920

(4) That to (b) now (c) for "Kjeldahl flask" substitute "200 cc. flask" and add "Generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meals and materials of like nature it is best to add about 5 cc. of strong nitric acid before the addition of the sulfuric acid, and allow to digest, at a gentle heat if necessary, until the violence of the reaction is over, before adding the nitrate".

(5) That to (c) now (e) add, "Suitable for materials containing small quantities of organic matter".

(The above changes were approved for first action of a change in an official method at the 1923 meeting.)

Approved (final action).

(2) That the action of the association at the 1923 meeting deleting the phrase in paragraph 6 "Determination," reading "Nearly neutralize with hydrochloric acid" and substituting therefor the sentence "Neutralize the cooled solution with hydrochloric acid using litmus as an indicator, and then add 1 cc. of strong hydrochloric acid" be rescinded and that the original wording as given in the present *Book of Methods* be restored and inserted in the revision now being made.

Approved under suspension of the rules.

(3) That paragraph 8 "Preparation of Solution" be changed to correspond with the change made in paragraph 5 by substituting the following, "Dissolve according to 5 (b), (c), or (d), preferably by (c), when these acids are suitable, and dilute to 200 cc. with water".

Approved (final action).

(4) That the action taken by the association at the 1923 meeting prefacing the text of paragraph 10, "Gravimetric Method.—Official", with the sentence "If mechanical condition makes necessary, triturate 2 grams of the sample with a small amount of water and wash on filter. Otherwise place etc." be rescinded and that the text of the present *Book of Methods* be followed in the revision now under way.

Approved under suspension of the rules.

(5) That the cross references in paragraph 13 "Determination" be changed to conform with the changes made in paragraph 5.

Approved (final action).

(6) That in paragraph 48 "Preparation of Solution" the cross reference 5 (g) be changed to 5 (d) to correspond with the change made in paragraph 5.

Approved (final action).

(7) That in paragraph 50 "Volumetric Method.—Official" the cross reference 5 (g) be changed to 5 (d) to correspond with the change made in paragraph 5.

Approved (final action).

## PHOSPHORIC ACID.

It is recommended—

(1) That collaborative study be made of the directions given in the official methods for determining the reaction of the solution to which magnesium mixture<sup>1</sup> is added in the gravimetric method for total phosphoric acid.

Approved.

(2) That a study be made of the use of acid magnesium mixture<sup>2</sup> used in the present official method.

Approved.

## NITROGEN.

It is recommended—

(1) That the Devarda alloy method for the determination of nitrogen in nitrate salts be continued as a tentative method.

Approved.

(2) That the use of sodium thiosulfate, instead of potassium or sodium sulfide, to precipitate the mercury in the Kjeldahl method and its modification be adopted as an optional procedure.

Approved (final action).

## POTASH.

It is recommended that a study be made of the precipitation of phosphoric acid by magnesium oxide and magnesium chloride in the technique of the Lindo-Gladding method for the determination of potash in mixed fertilizers.

Approved.

## PLANTS.

It is recommended that the work now under way be continued.

Approved.

## SULFUR AND PHOSPHORUS IN SEEDS OF PLANTS.

It is recommended—

(1) That the magnesium nitrate method for the determination of sulfur and phosphorus in plants<sup>3</sup> including the seeds of plants, as outlined in the report of the associate referee, be adopted as an official method. (Final action.)

Approved.

(2) That the magnesium nitrate method replace the present official method. (Final action.)

Approved.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 3 (6)

<sup>2</sup> *Ibid.*, 2 (4C)

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6 414

## REPORT OF SUB-COMMITTEE B ON RECOMMENDATIONS OF REFEREES, 1924.

By E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.),  
*Chairman.*

### CHEMICAL REAGENTS.

It is recommended that the observations on reagent chemicals be continued and that collaborative work be instituted if possible.

Approved.

### SPICES AND OTHER CONDIMENTS.

#### SALAD DRESSING.

It is recommended that the applicability of the present tentative methods for the determination of fat and lipoid phosphoric acid in eggs and egg products<sup>1</sup> to the determination of these constituents in salad dressings be further studied; and that the modifications embodied in the proposed methods, as outlined by the referee this year, be further studied.

Pending this further investigation no change in the classification of salad dressings is recommended.

Approved.

#### PREPARED MUSTARD.

It is recommended that the Hiltz-Hertwig method for the determination of crude fiber in prepared mustard<sup>2</sup> be adopted as official. (Final action.)

Approved.

### NAVAL STORES.

#### TURPENTINE OIL.

It is recommended—

(1) That the tentative fuming sulfuric acid method for mineral oil in turpentine oil<sup>3</sup> be adopted as an official method. (Final action.)

Approved.

(2) That final action on the sulfuric acid-fuming nitric acid method for mineral oil in turpentine oil be deferred.

Approved.

(3) That the recommendation made last year<sup>4</sup> that the Grotlisch-Smith method for coal tar oils in turpentine oil<sup>4</sup> be further studied, be repeated.

Approved.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 10-11; 1924, 7: 279.

<sup>2</sup> *Ibid.*, 1923, 7: 71; 1924, 7: 270.

<sup>3</sup> *Ibid.*, 1923, 6: 466; 1924, 7: 272.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1921, 13: 791.

## DRUGS.

## ACETYLSALICYLIC ACID.

It is recommended—

(1) That the iodine method for total salicylates<sup>1</sup>, now tentative, be adopted as an official method. (Final action.)

Approved.

(2) That Method II for the determination of combined acetic acid, as rewritten by the associate referee this year, be adopted as a tentative method.

Approved.

(3) That the tentative method<sup>2</sup> for determining acetylsalicylic acid in mixtures, as amended and rewritten by the associate referee this year, be adopted as an official method. (First action as an official method.)

Approved.

(4) That work on the determination of free acetic acid in acetylsalicylic acid be discontinued.

Approved.

(5) That further work be not undertaken by the referee upon the determination of acetylsalicylic acid in admixture with interfering substances (laxatives), as was contemplated in recommendation 3 last year<sup>3</sup>.

Approved.

(6) That the tentative method for the quantitative determination of free salicylic acid<sup>4</sup> be adopted as an official method. (First action as an official method.)

Approved.

(7) That the bromine method for the determination of total salicylates be given such study during the coming year as the associate referee may deem necessary for recommending the same as official.

Approved.

(8) That the double titration method for the determination of acetylsalicylic acid be studied by the associate referee in comparison with a simple saponification with alcoholic potash, and that recommendations be made for the proper disposition of the double titration method next year.

Approved.

(9) That the relative merits of the "dry" and "wet" methods of extracting acetylsalicylic acid with chloroform be studied and a uniform procedure recommended.

Approved.

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<sup>1</sup> *J. Asso. Official Agr. Chemists*, 1922, 5: 582.

<sup>2</sup> *Ibid.*, 1924, 8: 29.

<sup>3</sup> *Ibid.*, 7: 271.

<sup>4</sup> *Ibid.*, 1922, 5: 582.



ALCOHOL IN DRUGS.

It is recommended that the study of the determination of alcohol in drugs be continued during the coming year.

Approved.

ARSENICALS.

It is recommended—

(1) That Method II for the determination of arsenic in arsphenamine and neoarsphenamine<sup>1</sup> be adopted as official. (Final action.)

Approved.

(2) That methods for the determination of arsenic in sodium cacodylate be further studied, as suggested by the associate referee this year.

Approved.

PHENYLCINCHONINIC ACID (ATOPHAN).

It is recommended that the study of atophan be discontinued for the present in accordance with the suggestion of the referee.

Approved.

BARBITAL (VERONAL) AND PHENOBARBITAL (LUMINAL).

It is recommended—

(1) That the method for the estimation of barbital and phenobarbital, as described by the associate referee this year, be adopted as a tentative method.

Approved.

(2) That the procedure for melting point determination be adopted as a tentative method.

Approved.

(3) That further study of the qualitative methods be discontinued, as suggested by the referee.

Approved.

CAMPHOR AND MONOBROMATED CAMPHOR.

It is recommended—

(1) That the referee arrange for the appointment of an associate referee to study methods for the determination of camphor, and that the methods cited by Arner<sup>2</sup> and any other available methods be studied collaboratively.

Approved.

(2) That the referee on drugs arrange for the appointment of an associate referee to study methods for the determination of mono-

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6, 463.

<sup>2</sup> *Ibid.*, 1922, 5: 544.

bromated camphor, and that the present tentative methods<sup>1</sup> and any other available methods be studied collaboratively with samples of known composition.

Approved.

#### CHAULMOOGRA OIL.

It is recommended that the collaborative studies outlined by the associate referee this year be continued during the coming year.

Approved.

#### CHLORAMINE PRODUCTS.

It is recommended that the subject of chloramine products be studied during the coming year.

Approved.

#### CHLOROFORM.

It is recommended that study of the method for the determination of chloroform be continued in the light of the experience and suggestions reported by collaborators this year.

Approved.

#### IPECAC ALKALOIDS.

It is recommended that the study of ipecac alkaloids be continued.

Approved.

#### RADIO ACTIVITY IN DRUGS AND WATER.

It is recommended—

(1) That the method described in the report of the associate referee this year be adopted as tentative.

Approved.

(2) That the associate referee during the coming year (1) develop suitable methods for the preparation of miscellaneous samples for analysis, and (2) prepare a description of the preparation of a standard stock solution of radium.

Approved.

#### LAXATIVE AND BITTER TONICS.

It is recommended—

(1) That the gravimetric assay of cascara drug and fluid extract of cascara, as submitted by the associate referee, be not adopted as tentative this year, but submitted to further collaborative study.

Approved.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 587.

(2) That the colorimetric check method be further studied with a view to simplifying it and increasing its accuracy.

Approved.

#### MERCURIALS.

It is recommended that a further study be made of the method by Rupp<sup>1</sup> and that of Jamieson<sup>2</sup> for the determination of mercuric chloride in antiseptic tablets.

Approved.

#### METHYLENE BLUE.

It is recommended that the study of methylene blue be discontinued for the present, as suggested by the referee.

Approved.

#### PAPAIN.

It is recommended that work on papain be discontinued.

Approved.

#### PHENOLPHTHALEIN.

It is recommended—

(1) That Methods I (iodination method) and II (ether-extraction method), for the determination of phenolphthalein in tablets<sup>3</sup>, which were adopted as tentative last year<sup>4</sup>, be adopted as official. (First action as official methods.)

Approved.

(2) That the modifications of these methods for application to chocolate-containing products, as described by the associate referee, be adopted as tentative.

Approved.

#### PYRAMIDON.

It is recommended—

(1) That qualitative tests designated as 1, 4, 5, and 6<sup>5</sup>, which were adopted as tentative last year<sup>6</sup>, be adopted as official. (First action as an official method.)

Approved.

(2) That the quantitative methods, I (extraction method) and II (hydrochloride method), as rewritten by the associate referee this year, be adopted as tentative methods for the determination of pyramidon.

Approved.

<sup>1</sup> *Chem. Ztg.*, 1908, 32: 1077.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1919, 11: 296.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 14.

<sup>4</sup> *Ibid.*, 271.

<sup>5</sup> *Ibid.*, 30.

<sup>6</sup> *Ibid.*, 275.

(3) That a method for extracting pyramidon directly from the dry powder be studied.

Approved.

#### SEPARATION OF QUININE AND STRYCHNINE.

It is recommended—

(1) That the Bliss method be not adopted as an official or as a tentative method.

Approved.

(2) That the Simmonds method be further studied, together with any other available methods.

Approved.

#### SILVER PROTEINATES.

It is recommended that the methods proposed by the associate referee for the examination of silver proteinates be adopted as tentative.

Approved.

#### SANTONIN.

The recommendation made and approved last year, that the tentative method for the detection of santonin in wormseed be studied with the purpose of making it official, is repeated.

Approved.

#### NEW TOPICS FOR STUDY.

It is recommended—

(1) That study of the determination of nitroglycerine be undertaken.

Approved.

(2) That study of apomorphine be undertaken.

Approved.

(3) That methods for the determination of ether in drug products be studied.

Approved.

REPORT OF SUB-COMMITTEE C ON RECOMMENDATIONS  
OF REFEREES.

By W. C. GEAGLEY (Department of Agriculture, Lansing, Mich.),  
*Chairman.*

[Dairy products (moisture in cheese, fat in malted and dried milk), fats and oils, baking powders and baking chemicals (fluorides in baking powder), eggs and egg products (liquid and frozen eggs, dried eggs, zinc in dried eggs), food preservatives, coloring matters in foods, metals in foods (arsenic), fruits and fruit products (pectin in jams, jellies, and preserves, fruit acids), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins), gelatin, cereal foods (moisture, ash, chlorine in bleached flour, glutenin in flour, methods for sampling flour), cacao products (microscopical methods, crude fiber, cacao butter).]

## DAIRY PRODUCTS.

## BABCOCK METHOD.

It is recommended—

(1) That the Babcock Method<sup>1</sup> as submitted by the referee at the 1923 meeting be adopted as an official method. (Final action.)

Approved.

(2) That the directions<sup>2</sup> for collection and preparation of samples under the heading "milk" and under the heading "cream" be made official. (Final action.)

Approved.

(3) That the following tentative methods in Chapter XXI, "Dairy Products"<sup>3</sup>, be made official (final action):

(1) Section 17, in paragraphs (a) and (b), "Sour Serum"; (2) Section 18, "Zeiss Refractometer Reading of Copper Serum"; (3) Section 19, "Gelatin"; (4) Section 21, "Coloring Matters"; (5) Section 28, "Gelatin"; (6) Sections 38 and 39, "Sucrose"; (7) Section 50, "Coloring Matters"; (8) Section 62, paragraphs (a) and (b), "Examination of Fat"; (9) Section 64, "Preparation of Sample"; (10) Section 65, "Roese-Gottlieb Method".

Inasmuch as the above-mentioned tentative methods have been used satisfactorily for a long time and are now in general use it is the recommendation of the committee that the by-laws be suspended, and that these methods be adopted as official. (Final action.)

Approved.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 9

<sup>2</sup> *Ibid.*, 7: 276.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 229-236.

(4) That the methods for the determination of "Specific Gravity" and "Acidity", as given by the referee in his report, be adopted as tentative methods.

Approved.

(5) That under the heading "Malted Milk" the methods for "Preparation of Sample", "Moisture", "Protein", "Ash", and "Fat" be adopted as tentative methods.

Approved.

(6) That the referee study these and other methods for the examination of malted milk during the coming year.

Approved.

(7) That Section 61, "Babcock Method.—Tentative", of Chapter XXI "Dairy Products"<sup>1</sup>, under the heading "Cheese", be deleted.

Approved.

(8) That Section 66, "Harding-Parkin Method.—Tentative", of Chapter XXI, "Dairy Products"<sup>2</sup>, under the heading "Ice Cream (Plain)", be deleted.

Approved.

#### MOISTURE IN CHEESE.

It is recommended—

(1) That the present tentative method<sup>3</sup> and the proposed vacuum<sup>4</sup> method for the determination of moisture in cheese, described by the associate referee in his report, be subjected to further comparative study.

Approved.

(2) That in view of the difficulties in obtaining uniform samples, each collaborator be requested to make studies on samples prepared by himself.

Approved.

#### FAT IN DRIED MILK.

It is recommended—

(1) That the Roesse-Gottlieb method for the determination of fat in dried milk, described in the report of the associate referee, be adopted as a tentative method and subjected to further study.

Approved.

(2) That the method for the determination of moisture in dried milk, described by Holm<sup>5</sup>, be subjected to collaborative study.

Approved.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 236.

<sup>2</sup> *Ibid.*, 237.

<sup>3</sup> *Ibid.*, 234.

<sup>4</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5 498.

<sup>5</sup> *Ibid.*, 509.

## FATS AND OILS.

It is recommended—

(1) That the Kerr-Sorber method<sup>1</sup> for the determination of unsaponifiable matter be adopted as an official method. (First action as an official method.)

Approved.

(2) That further work be done on the determination of unsaponifiable matter.

Approved.

(3) That the study on the determination of acetyl value<sup>2</sup> be continued.

Approved.

(4) That the heading for Section 2 of Chapter XXII, Fats and Oils, "Specific Gravity at 20°/40°C.—Official"<sup>3</sup> be changed to read "Specific Gravity at 25°/25°C.—Official", and that the text of the directions be changed to provide that the determination be made at 25°/25°C. instead of 20°/40°C.

Approved.

## BAKING POWDERS AND BAKING CHEMICALS.

It is recommended—

(1) That Section 2, "Total Carbon Dioxide, General Method—Tentative", of Chapter XXVII, "Baking Powders and Baking Chemicals"<sup>4</sup>, be deleted.

Approved.

(2) That the official method for acidity<sup>5</sup>, Section 11, be amended by substituting the words "sodium hydroxide" for the words "potassium hydroxid".

Approved, final action, under suspension of the rules.

(3) That Section 15, "Free Tartaric Acid" and Section 16, "Potassium Bitartrate" of Chapter XXVII "Baking Powders and Baking Chemicals"<sup>6</sup> be deleted.

Approved.

(4) That additional collaborative data be secured on the electrolytic determination of lead in baking powder.

Approved.

(5) That further collaborative studies of the gasometric method<sup>7</sup> for the determination of carbon dioxide be made in which the determinations are made at approximately the same time, preferably immediately after receipt of samples.

Approved.

<sup>1</sup> *Cotton Oil Press*, 1924, 7: 40.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 277.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 239.

<sup>4</sup> *Ibid.*, 277.

<sup>5</sup> *Ibid.*, 281.

<sup>6</sup> *Ibid.*, 282.

<sup>7</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 453.

(6) That studies be undertaken to develop a method for determining the neutralizing value of mono-calcium phosphate that will show the exact quantity of bicarbonate of soda required.

Approved.

#### FLUORIDES IN BAKING POWDER.

It is recommended that further collaborative and experimental studies be made of the method<sup>1</sup> for the determination of fluorine in baking powder.

Approved.

#### EGGS AND EGG PRODUCTS.

It is recommended—

(1) That the methods for the taking and preparation of samples of liquid, frozen, and powdered dried egg, described by the referee in his report, be adopted as tentative methods.

Approved.

(2) That the referee study the preparation of samples of flaked dried eggs, and that he include in this study the methods suggested in the report of the referee.

Approved.

(3) That the method for the determination of total solids in liquid eggs and powdered dried eggs, described by the referee in his report, be adopted as official. (First action as an official method.)

Approved.

(4) That the referee study a rapid method for the determination of total solids in eggs similar to the rapid method recommended for determining moisture in flour at 130°C. and at atmospheric pressure.

Approved.

(5) That the method for the determination of ash in eggs be studied, and that attention be given to the type of material of the ashing dish, as platinum may be injured by the high phosphorus content of the egg.

Approved.

(6) That the method for the determination of organic and ammoniacal nitrogen in powdered dried eggs and liquid eggs, described by the referee in his report, be adopted as official. (First action as an official method.)

Approved.

(7) That the method for the determination of fat in liquid and powdered dried eggs, described by the referee in his report, be adopted as tentative.

Approved.

(8) That the method for the determination of lipoids and lipid phosphoric acid ( $P_2O_5$ ) in liquid eggs and powdered dried eggs, described by the referee in his report, be adopted as tentative.

Approved.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 457.



(9) That the method, including the modifications suggested by the referee in his report, for the determination of water-soluble protein precipitable be studied during the coming year.

Approved.

(10) That the study of the determination of zinc in eggs be continued during the coming year.

Approved.

(11) That methods for determining unsaponifiable matter and sterols in egg products be studied during the coming year.

Approved.

(12) That a study of the method for the determination of acid-soluble phosphoric acid in eggs be continued, and that in this connection the suggestions made by the referee be given consideration.

Approved.

(13) That a study of the method for acidity of fat in eggs be continued, and that the suggestions made by the referee be given consideration.

Approved.

(14) That the methods for the analysis of egg noodles, adopted as tentative at the 1923 meeting, be included in the chapter entitled "Cereal Foods" instead of in the chapter entitled "Eggs and Egg Products"<sup>1</sup>, as was previously recommended.

Approved.

#### FOOD PRESERVATIVES.

It is recommended that a study be made of the ease and accuracy of the present official methods for the determination of salicylic acid, benzoic acid or benzoates, and saccharin in food stuffs, in comparison with modified methods involving the use of the sublimator.

Approved.

#### COLORING MATTERS IN FOODS.

It is recommended—

(1) That the methods for the separation and identification of the recently permitted coal tar food colors Light Green SF Yellowish, Guinea Green B, Yellow AB, and Yellow OB, be adopted as tentative and incorporated in the revised chapter of methods, "Coloring Matters in Foods".

Approved.

(2) That collaborative studies be made of the methods for the separation and identification of the permitted coal tar dyes described by the referee in his report.

Approved.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 279.

(3) That further studies be made of methods for the separation of Yellow AB and Yellow OB from other oil-soluble dyes.

Approved.

(4) That further studies be made on the "red phase" of Yellow AB.

Approved.

#### METALS IN FOODS.

No report was submitted by the referee.

It is recommended that the zinc-iron precipitation method for tin<sup>1</sup> be studied collaboratively in comparison with the Baker-Sellars method<sup>2</sup> with a view to making one or both of these methods official.

Approved.

#### ARSENIC.

It is recommended—

That the concentration of acids used in the official method for the determination of arsenic<sup>3</sup> be changed to read as follows:

Section 1 (c): Dilute one volume of the arsenic-free concentrated acid with four volumes of water.

Section 1 (d): Dilute one volume of the arsenic-free hydrochloric acid with three volumes of water.

Approved, final action, under suspension of the rules.

#### FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That the Kling method for the determination of tartaric acid, as described by the referee in his report<sup>4</sup>, be adopted as tentative.

Approved.

(2) That further study of the official method for the determination of commercial glucose<sup>5</sup> in jams, jellies, and preserves be made.

Approved.

(3) That a study of the determination of malic acid in the presence of citric and tartaric acids be undertaken.

Approved.

(4) That methods for the determination of fruit ash in the case of fruit products containing added non-volatile ingredients like phosphoric acid, alum, or calcium salts be further studied.

Approved.

(5) That the refractive index method for total solids in fruit products be further studied with a view to substituting it for the drying method.

Approved.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 29.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 150.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 272; 1924, 8: 280.

<sup>4</sup> *Bull. Soc. Chim.*, 1910, 7: 567; 1912, 11: 886.

<sup>5</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 155.

(6) That the procedure for determining added water in white grape juice, as described in the report of the referee, be adopted as tentative.

Approved.

(7) That the method for determining added water in grape juice be presented for collaborative work during the coming year.

Approved.

(8) That the official method for tartaric acid be made the subject of further study for the purpose of improving its accuracy.

Approved.

#### CANNED FOODS.

It is recommended that the referee continue the study of methods for detecting spoilage in the different varieties of canned foods.

Approved.

#### VINEGARS.

No report was submitted by the referee.

It is recommended that the method for polarization using decolorizing carbons<sup>1</sup> be further studied.

Approved.

#### FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the Folin and Denis rapid colorimetric method for the determination of vanillin in vanilla extract and its imitations, described by the referee in his report, be adopted as an alternative official method. (First action as an official method.)

Approved.

(2) That the Wichmann method for the determination of the lead number of vanilla extract and its imitations, described by the referee in his report, be adopted as an alternative official method. (First action as an official method.)

Approved.

(3) That the chromate method for the determination of lead, described by the referee in his report, be adopted as an alternative official method. (First action as an official method.)

Approved.

(4) That the words "of lemon" be deleted from the sentence "If oil of lemon is present in amounts over 2 per cent \* \* \*" in the present official method<sup>2</sup> for the determination of oil in lemon and orange extracts. (First action for a change in an official method.)

Approved.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 150.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 200.

(5) That the Hortvet and West method<sup>1</sup> for the determination of alcohol in extracts consisting only of oil, alcohol, and water, be adopted as an alternative official method. (Final action. First action was taken in 1919.)

Approved.

(6) That the incoming referee continue to clear away the old, unacted-upon recommendations listed in the report of the referee.

Approved.

(7) That to avoid confusion the present official method for lead number be designated as "Lead Number (Winton)".

Approved.

#### MEAT AND MEAT PRODUCTS.

It is recommended—

(1) That the present tentative method<sup>2</sup> for the determination of sugar in meats be subjected to study for the possible detection and correction of any faults contained therein, and that an effort be made to develop a new method for the determination of sugar in meats.

Approved.

(2) That the method for the determination of nitrites in cured meat, as described in the report of the referee, be adopted as a tentative method.

Approved.

#### SEPARATION OF MEAT PROTEINS.

No report was submitted by the referee.

It is recommended that cooperative work be done on the modified Van Slyke methods for the determination of amino acids in the globulin-albumin fractions of beef flesh that were presented to the association at the 1921 meeting<sup>3</sup>.

Approved.

#### GELATIN.

No report was submitted by the referee.

It is recommended that the incoming referee continue the study of methods for the determination of copper and zinc.

Approved.

#### CEREAL FOODS.

It is recommended—

(1) That the referee study methods for taking and preparing for analyses samples of flour.

Approved.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 308; *J. Ind. Eng. Chem.*, 1909, 1: 94.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 213.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 86.

(2) That the official method for the determination of moisture in flour<sup>1</sup> be amended by deleting the phrase "in a current of hydrogen or".

Approved.

(3) That the vacuum method for the determination of moisture in flour, described by the referee in his report, be subjected to further collaborative study.

Approved.

(4) That the rapid method for determining moisture in flour, as described by the referee in his report, be subjected to further study during the coming year.

Approved.

(5) That the method for the determination of ash in flour<sup>2</sup>, described by the referee in his report, be adopted as an official method. (Final action.)

Approved.

(6) That Section 2<sup>3</sup> "Ash" under the heading "Wheat Flour", Chapter XIV "Cereal Foods", be deleted.

Approved.

(7) That the rapid methods for determining ash in flour, described by the referee in his report, be subjected to further studies during the coming year.

Approved.

(8) That the referee study methods for the determination of glutenin in flour during the coming year.

Approved.

(9) That the methods "Fat (Acid Hydrolysis Method)" and "Lipoids and Lipoid Phosphoric Acid ( $P_2O_5$ )" for the examination of flour, described by the referee in his report<sup>4</sup>, be subjected to collaborative study.

Approved.

(10) That the method for the determination of "Water-Soluble Protein—Nitrogen Precipitable by 40% Alcohol", described by the referee in his report<sup>5</sup>, be subjected to collaborative study.

Approved.

(11) That the method submitted by the associate referee for the determination of chlorine in bleached flours be studied during the coming year.

Approved.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 167.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 132.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 167.

<sup>4</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 508.

<sup>5</sup> *Ibid.*, 7: 84.

(12) That studies be undertaken of methods for the examination of bread, which studies shall include the association methods for wheat flour and alimentary pastes, in so far as they are practicable.

Approved.

(13) That the determination of moisture in alimentary pastes be further studied.

Approved.

#### CACAO PRODUCTS.

##### MICROSCOPICAL EXAMINATION.

It is recommended—

(1) That the study of methods for the estimation of shell in cacao products be continued.

Approved.

(2) That the study of methods for the detection of foreign fats in cacao products containing milk be continued.

Approved.

(3) That the crude fiber content of alkali-treated cacao products be studied.

Approved.

(4) That the methods for the determination of fat in cacao products as proposed by Lepper and Waterman and by Feldstein be studied collaboratively.

Approved.

### THIRD DAY.

## WEDNESDAY—AFTERNOON SESSION.

### REPORT OF COMMITTEE TO COOPERATE IN REVISION OF THE U. S. PHARMACOPEIA.

No date has been announced for the appearance of the Tenth Decennial Revision, but many monographs in galley form have been read by your chairman and other members of the committee and various suggestions have been offered. It is reasonably certain that the book will be published within the next year.

It seems desirable to call attention to several features identified with this book of legal standards. The Pharmacopeia is essentially a compilation of analytical methods for establishing certain drug standards. This is the part that primarily concerns the A. O. A. C. The book was started one hundred years ago by physicians who early realized the need of pharmaceutical cooperation. In time the pharmacists domineered several revisions, and the physicians seemed to lose interest and in fact severely neglected their child. Things have materially changed. The present revision committee represents physicians, pharmacists, chemists, botanists, pharmacognosists, pharmacologists, microscopists, manufacturers, etc. Each group is assigned to its especial task and, it is believed, with excellent results. The physicians say what drugs are to be included and decide all therapeutic questions, while the other workers handle the remaining features. The new Pharmacopeia will undoubtedly recognize the most valuable medicinal agents and the best methods for establishing their identity, quality, and purity.

About 650 articles have been admitted. Among the new titles added may be mentioned the following: Acetylsalicylic Acid, Amidopyrine, Strong Silver Protein, Mild Silver Protein, Arsphenamine, Barbitol Sodium, Barium Sulfate, Ethyl Aminobenzoate, Carbon Tetrachloride, Dichloramine, Epinephrine, Chaulmoogra Oil, Phenolsulfonephthalein, Procaine Hydrochloride, Spiritus Frumenti, Spiritus Vini Gallici, and Thyroxin.

The medical profession needs reliable medicines and is now asking to be provided with drugs of dependable quality; to meet this request effort should be made to introduce more methods of standardization for drugs included in the Pharmacopeia. Although it has been recognized generally that biological assay methods are among the most important,

no satisfactory procedure exists for producing uniform results. It is believed that the problem has been solved, in part at least, since the present committee of revision has arranged with the Bureau of Chemistry to supply manufacturers with type specimens of certain drugs to be used for standardization work. This will undoubtedly make for greater dependability and uniformity.

It is now recognized that vitamins play a very important part in the health and welfare of humanity. Since cod liver oil contains a goodly proportion of vitamin A, an effort is being made to devise a method for standardizing this useful drug on the basis of vitamin content. The method may be optional, but the probabilities are that some method will be recognized.

The details for identifying many inorganic chemicals will be placed in a special chapter in Part 2, and therefore they will be only briefly referred to under the chemicals themselves.

The members of the association who are called upon to sample drugs know the difficulties attending this work and will welcome the announcement that standardized methods of sampling are to be included in the present revision. The standards for whiskey and brandy provided will include tests for those denaturing substances employed for making industrial alcohol, such as methyl alcohol and diethylphthalate. The "bottled in bond" label is not to be required, which of course necessitates more extensive tests. "Regulation 60" of the Treasury Department makes "bottled in bond" goods obligatory for prescription purposes.

An organoleptic test for tincture and oleoresin of capsicum will be included. It prescribes that one part of the drug in 7000 parts of a sweetened aqueous solution "should produce a distinct sensation of pungency and the taste of capsicum in the mouth and throat".

Proximate assay methods include both total extraction and aliquot part processes, and volumetric and gravimetric estimations of the alkaloids. The use of either cochineal or methyl red as indicator for alkaloidal work is permitted.

Details are given for extracting the active principles of ground drugs, tinctures, powdered extracts, fluid extracts, pilular extracts, plasters, liniments, etc.

It is recommended that the committee be continued for another year.

L. F. KEBLER,	A. R. BLISS,
H. C. LYTHGOE,	J. M. DORAN.
H. C. FULLER,	

*Committee to Cooperate in Revision of the  
U. S. Pharmacopeia.*

**Approved.**



REPORT OF THE REPRESENTATIVES OF THE A. O. A. C. ON  
THE BOARD OF GOVERNORS OF THE CROP PRO-  
TECTION INSTITUTE OF THE NATIONAL  
RESEARCH COUNCIL.

Representatives: BURT L. HARTWELL and H. J. PATTERSON.

Report made by H. J. PATTERSON.

MR. PRESIDENT, LADIES, AND GENTLEMEN: I did not expect to be called upon for this report as Mr. Hartwell, the chairman, has performed this duty in previous years. What I shall say must necessarily be impromptu.

Since our last report your representatives have attended two meetings of the Crop Protection Institute Board, one at Cincinnati during the A. A. A. S. sessions in December, 1923, and the annual meeting in New York on February 14, 1924.

The need for an organization such as the Crop Protection Institute has been proved, as is evidenced by the increasing number of industries that are using the Institute for investigating fundamental problems confronting them and for determining the real worth of various agencies that they are manufacturing for use in protecting crops from diseases or insect ravages or in preventing such diseases.

While most of these projects are more closely related to the work of the entomologist and plant pathologist than to that of the chemist, yet their fundamental solutions are needing more and more the help of the chemist. Plant protection problems in many instances depend almost wholly on the chemistry of the product used and its physiological effect on the crop. This means that the entomologist, the plant pathologist, the chemist, the bio-chemist, and the plant physiologist must cooperate in solving problems, and that investigations must be organized around the project rather than by departments. I believe that you can all appreciate that the chemist has a big interest in an organization such as the Crop Protection Institute and should have a voice in shaping its policies and plans and outlining and supervising many of its projects. This, I am pleased to report, seems to be fully recognized by the officers and Board of Governors of the Institute.

The principal projects now under investigation and supervision by the Institute relate to the following substances: sulfur, nickel, furfural, scalecide, and calcium arsenate.

Negotiations for several other projects are under consideration.

All these investigations are financed by commercial organizations. They are planned and supervised by specialists who seem best qualified for the work. The work is done at existing institutions where the best

facilities can be offered, and most of it is performed by persons hired for the particular project and who devote their full time to it.

The results are issued by the Institute and published in existing journals. Results that are patentable are patented in the name of the Institute and remain the property of the Institute after the general plans adopted by the National Research Council.

Any member of this association who knows of any industry needing the help of a neutral organization for conducting its research or testing its products would do the Crop Protection Institute a service by calling it to attention.

The Crop Protection Institute has no paid officers. The officers, Board of Governors, and supervisors of investigations all give their time gratuitously.

I regret that Mr. Hartwell, the chairman of the committee, is not present to give this report as I am sure he would have made it more worth while.

## REPORT OF THE SECRETARY-TREASURER.

By W. W. SKINNER (Bureau of Chemistry, Washington, D. C.).

The number of institutions paying membership dues has been increasing gradually. Sixty-nine memberships were recorded in 1924 compared with 52 in 1922 and 60 in 1923. The bank balance increased from \$414.54 to \$685.54 this past year. Every effort has been made, as usual, to keep the expenditures at a minimum until *The Journal* is running on a self-supporting basis. It is encouraging to note, in this our 40th anniversary year, that this goal is becoming less visionary than it has ever been.

No unusual requests have come to the attention of the secretary, but numerous inquiries of a general nature have been received and answered. These inquiries generally relate to specific methods, to the policies of the association, or to matters that are referred to offices of the Department of Agriculture. The idea seems to prevail that this association is conducting a general technical book business in addition to its own publications.

It is with regret that it is necessary to record the deaths during the past year of four members of the association. Dr. William Alphonso Withers, Dr. W. C. Stubbs, Mr. R. W. Hilts, and Mr. A. W. Ogden. Dr. Withers was Professor of Chemistry at the Agricultural and Mechanical College of North Carolina from the founding of that institution in 1889 to the time of his death, and served as president of this association in 1909-10; Dr. Stubbs was a former State Chemist of Louisiana and also Director of the Experiment Stations in Louisiana; Mr. Hilts was Chief of the Western Food and Drug Inspection District of the Bureau of Chemistry; and Mr. Ogden was at one time chemist in the Agricul-

tural Experiment Station at New Haven, Conn., and later a member of the staff of the New York Food and Drug Inspection Station of the Bureau of Chemistry.

In preparing for Dr. Wiley's anniversary dinner, the committee had occasion to make a search for photographs of the association taken in the earlier years of its existence. This effort led to expressions by the members of the committee as to the desirability of having a file of the group pictures as well as of those of all past presidents of the association. The secretary will appreciate every effort on the part of the members to assist in making such a collection complete.

The following changes of refereeships were made during the year: W. H. Ross was appointed Associate Referee on Phosphoric Acid to take the place of R. B. Deemer, and William Seaman became Associate Referee on Honey to succeed S. F. Sherwood.

At the meeting of the Executive Committee held this week considerable time was devoted to a discussion of the revision of the *Book of Methods*. It was decided that all tables should appear in the back of the book and not be distributed throughout the text. It was decided to omit all advertising matter.

The committee decided to appoint an associate referee on methods for the analysis of ice cream, the associate referee to serve under the General Referee on Dairy Products. The committee also decided that this association should continue its cooperation with the American Public Health Association in the study of methods for milk analysis, with the understanding that the chemical methods shall be those of the A. O. A. C.

The committee approved the suggestion of the Chairman of the Board of Editors to change the volume date of *The Journal* so as to have it correspond with the calendar year. The committee also approved the suggestion of the editor to furnish 50 reprints free to authors of contributed articles, and the recommendation that each general referee suggest to the committee an associate referee on methods of sampling was accepted.

The financial statement will be found on page 246.

## REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

This committee respectfully submits the following report covering the proceedings of the Joint Committee on Food and Drug Definitions and Standards during the period following the last meeting of this association.

The committee held two meetings in the past year, one during the week beginning February 25th and another during the week beginning August 18th. The February meeting was devoted largely to a further discussion and revision of the tentative schedule of definitions for meat

products, and to a lengthy consideration of attempted definitions for plain ice cream. A tentative definition and standard for ice cream was adopted for publication in order to elicit criticisms and suggestions from the manufacturers, the State food enforcement officials, and the public in general. In the meat schedule substantial agreement was reached regarding the majority of the definitions, and it was hoped that the schedule would be in shape for approval during the next meeting of the committee. Considerable time was also devoted to a discussion of proposed definitions for different varieties or grades of flour, including the general term "wheat flour" and such well-known commercial names as "straight flour", "patent flour", and "clear flour". No definite conclusion was reached regarding these products, and the schedule was left in tentative shape for further consideration. The jams and jellies definitions and standards constituted also a subject for brief discussion. A conference was held with the Pea Cannery Committee of the National Cannery Association on the subject of definitions and standards for canned peas. A number of difficulties have arisen in connection with attempts to formulate satisfactory descriptions of grades that are claimed to be well recognized in the industry. The canners appear to be very strongly in favor of some action on the part of our committee, but on the other hand the committee is confronted in this particular instance with unusual difficulties in devising terms that will differentiate satisfactorily and substantially the various grades. It is desirable, of course, that any definitions that may be adopted shall be serviceable under the administration of the Federal and State pure food laws. A conference was also held with individuals representing manufacturers of alkalized or Dutch process chocolate and cocoa. Much additional valuable information was obtained, and a sub-committee was instructed to prepare a proposed schedule for these products to be submitted to the members of the committee for consideration. The subject of pie definitions also demanded some attention, chiefly with reference to a standard applicable to varieties of fruit pie. No definite action was taken; nevertheless it seems to be the sentiment of the committee that its members continue to devote their attention to this product in a gastronomic manner, as heretofore. Proposed changes in the definitions and standards for thyme and celery seed were given due consideration. After some discussion and the examination of data submitted by representatives of the Bureau of Chemistry definitions were adopted for cumin seed and marjoram, as follows:

#### CUMIN SEED.

*Cumin Seed* is the dried fruit of *Cuminum cyminum* L. It contains not more than nine and five-tenths per cent (9.5%) of total ash, not more than one and five-tenths per cent (1.5%) of ash insoluble in hydrochloric acid, nor more than five per cent (5%) of harmless foreign matter.

**MARJORAM, LEAF MARJORAM.**

*Marjoram, Leaf Marjoram*, is the dried leaves, with or without a small proportion of the flowering tops, of the *Majorana hortensis* Moench. It contains not more than sixteen per cent (16%) of total ash, not more than four and five-tenths per cent (4.5%) of ash insoluble in hydrochloric acid, nor more than ten per cent (10%) of stems and harmless foreign material.

The meeting held in August was signallized by a further consideration of a number of important subjects, chief among which were the ice cream definitions and the meat schedule. An afternoon conference with representatives of the National Association of Ice Cream Manufacturers resulted in securing a considerable amount of first-hand information for the committee. It was gratifying to learn that the manufacturers appeared to be unanimously in favor of the adoption not only of a definition for ice cream but also of a standard for fat. Some disagreements arose regarding the proposed stipulation relating to weight per gallon, and also regarding the use of coloring matter and other minor details of the proposed definition. This subject received further discussion during the convention of the American Dairy, Food, and Drug Officials held at Chattanooga during the following week, and the views of the delegates of the various States represented were thoroughly sounded out, particularly with reference to the standard for fat in plain ice cream. The manufacturers advocated an 8 per cent standard, while the majority of the opinions expressed by the food officials seemed to favor a standard not lower than 10 per cent or possibly the adoption of the standard of 12 per cent as in the proposed definition.

The committee held a further conference on the meat schedule with John R. Mohler and his associates of the Bureau of Animal Industry. The entire schedule was considered in detail in the order of the paragraphs, and special attention was given to a proposed definition for a class of sausages made with the addition of cereal. This variety of sausage has heretofore been subjected to regulatory control, but it was proposed, in view of the fact that these products have acquired an important status in the retail markets in the District of Columbia and elsewhere, that a definition be adopted which will place them on a more substantial basis for control under the operation of the food laws. Sometime during the past year the attention of the committee was called to a product known over a wide section of the country under the name "Sweet Cream Butter", and representations were made which seemed to justify an attempt to define this product as a distinct variety of butter. After some discussion a tentative definition was drawn up for the purpose of securing the views and criticisms of the manufacturers. The subject of alkalized or Dutch process cacao products was again taken up for discussion with the result that a tentative definition and standard was adopted in form to be submitted to the trade. A discussion of the flour schedule was resumed at the point

where it was dropped at the previous meeting, and a schedule in tentative form was drawn up to serve as a basis for consideration at the next meeting. A further discussion was held on the subject of jams and jellies, but no final action was taken. A conference was held with representatives of the Bureau of Chemistry on the subject of almond paste and almond cream, with the result that tentative definitions for these products were prepared for the purpose of discussion and probable final action at the next meeting.

JULIUS HORTVET,                      C. D. HOWARD.  
E. M. BAILEY,

*Committee to Cooperate with Other Com-  
mittees on Food Definitions.*

Approved.

## REPORT OF THE COMMITTEE ON SAMPLING.

At last year's meeting the association provided for the appointment of a committee to study methods of sampling<sup>1</sup>.

The importance of proper and well-carried-out methods of sampling is a subject that hardly needs elaboration. When one considers the fact that no matter how accurate analytical methods may be, or how accurately these methods may be followed, the results of such analyses, unless the sample on which the analyses are made is truly representative of the material sampled, will be largely vitiated and may even be valueless. When one further considers the importance attaching to analyses of samples both in the large numbers of daily business transactions based on such analyses as well as the legal aspects of food and drug analyses from the standpoint of enforcement laws, one is impressed at once with the absolute need for the development of methods of sampling that give an accurate and correct sample of the product in question and at the same time secure such samples in the most practical way. Baillie in a paper entitled "Some Problems and Fallacies of Sampling"<sup>2</sup> has aptly defined sampling as "that process whereby a representative and comparatively small portion of material is selected from a large bulk, and preserved under such conditions as to prevent contamination or avoidable alteration with a view to the formation of valid conclusions regarding the suitability of the bulk for specified purposes". This definition, while broad in character, is necessarily so on account of the need for precautions for the proper handling and preparation of the sample from the time of taking until its actual handling by the analyst making the examination.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 291.

<sup>2</sup> *Chemical Trade Journal*, 1920, 66: 333.

Much time and effort has been expended in the development and testing of methods of sampling as applied to such products as coal, minerals, ores, metals, oils, soaps, and waters. A number of these studies and reports have been presented by technical groups directly associated with the production and distribution of the products mentioned. Unfortunately, much less work has been done in the field of proper sampling of foods and drugs.

At the Fourth International Congress of Applied Chemistry held at Paris in 1900, provision was made for the appointment of an International Committee on Analysis. For various reasons this committee was unable to organize until November, 1902<sup>1</sup>. At the Berlin meeting a series of by-laws for the conduct of the committee's work was adopted, providing for the appointment of this committee by each International Congress of Applied Chemistry and that its members should hold office until the close of the next session. The object of this committee as expressed in the by-laws is as follows: "The object of the Committee is to bring about an international understanding: 1. As to the analytical methods to be applied in cases of dispute, primarily in disputes between parties belonging to different nations, leaving to the chemists of each nation the power to employ any other methods they choose for their own use. 2. As to tables, measuring instruments and any other auxiliaries in the same compass as for the analytical methods themselves. 3. As to other objects of interest for technical chemical analyses when required".

In the report of this committee to the Sixth International Congress of Applied Chemistry<sup>2</sup> is given a complete list of the various sub-committees of this international committee, together with the question or questions under consideration by these various sub-committees. Sub-committee No. 4 was charged with a study of the principles that should be observed in the sampling of materials for analysis. The report<sup>3</sup> of this sub-committee, of which Harvey W. Wiley was chairman, was divided into two parts, the first dealing with general principles of sampling and the second part dealing with the sampling of special classes of materials including soils, fertilizers, minerals containing fertilizing materials, mixed fertilizers, materials for road building, food products including butter, milk, cheese, sugar and molasses, cattle feeds, insecticides, drugs, chemicals, and crude vegetable drugs. A further report<sup>4</sup> was submitted by this same sub-committee under the chairmanship of Dr. Wiley to the Eighth International Congress of Applied Chemistry under the general title "General Methods of Sampling Especially as Applied to Chemical Products". This report quotes in full papers on

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<sup>1</sup> Fifth International Congress of Applied Chemistry, Berlin, June 2-8, 1903, 4: 949-56.

<sup>2</sup> Sixth International Congress of Applied Chemistry, Rome, 1907, 7: 25.

<sup>3</sup> *Ibid.*, 169-193.

<sup>4</sup> Report of the International Committee on Analyses to the Eighth International Congress of Applied Chemistry, 1912, pp. 42-69.

the "Sampling of Coal in the Mine", by Joseph A. Holmes<sup>1</sup>; "Methods of Sampling Deliveries of Coal as Practiced by the U. S. Bureau of Mines", by G. S. Pope<sup>2</sup>; and "The Methods of the United Steel Corporation for the Commercial Sampling of Iron Ores", by James M. Camp<sup>3</sup>.

In 1919 the Central Inspection District of the Bureau of Chemistry submitted an elaborate report on sampling which, besides outlining the fundamental considerations involved in sampling, also considered the most desirable methods for a series of individual food and drug products, as well as a consideration of the preparation for analysis of samples so collected. Unfortunately, this report has not been published.

In the development of any plans of work for the proper sampling of foods, drugs, and agricultural products considered by this association, certain general and fundamental considerations must be followed in any experimental study which may be undertaken. These considerations are as follows:

1. The sample obtained must be as nearly representative of the entire parcel sampled as is practicable.
2. A sufficient number of packages or other units must be collected to satisfy the principle laid down in Paragraph 1. Here, also, the purpose for which the sample is collected must, of course, be taken into consideration. Such samples are usually collected for one of the following three purposes:
  1. Chemical or bacteriological analysis.
  2. Net weight determination.
  3. Labeling practice.
- Again, where spoilage is involved, separate and special samples are required.
3. Precautions must be taken to prevent chemical and bacteriological changes during and after the collection of the sample.
4. The fineness of subdivision, or the state of aggregation of the sample to be collected, must be considered in the development of the most desirable method.
5. The application of hand or mechanical methods of collection of samples.
6. The preparation, packing, and shipment of samples.

#### RECOMMENDATIONS.

Therefore, in the opinion of this committee, the commercial and regulatory importance of adequate sampling justifies the following recommendations:

1. That associate referees on sampling and methods of sampling be appointed for the various types of products now considered by the association in its *Book of Methods*.
2. That such referees study the subject with special reference to the foregoing basic principles.
3. That sufficient collaboration be secured to furnish an adequate basis for a judgment as to the proper evaluation of the proposed methods.

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<sup>1</sup> U. S. Bur. Mines Technical Paper No. 1.

<sup>2</sup> U. S. Bur. Mines Bull. 11.

<sup>3</sup> J. Ind. Eng. Chem., 1909, 1: 107.



4. That following the development and acceptance by the association of such methods, they be incorporated in the association's methods of analysis.

5. That whenever possible, cooperative arrangements be made by such associate referees with the various industries involved, as well as with any commercial group of chemists connected with such industries, looking toward their active cooperation in the development of the most desirable methods of sampling.

F. C. BLANCK,	R. N. BRACKETT,
JAMES W. KELLOGG,	F. W. ZERBAN,
ARTHUR E. PAUL,	R. W. FREY,
A. G. MCCALL,	J. W. SALE,
C. C. McDONNELL,	A. J. PATTEN.

*Committee on Sampling.*

Approved by the association and referred to the Executive Committee with power to act.

#### REPORT OF THE SPECIAL COMMITTEE ON THE COLLABORATIVE STUDY OF METHODS OF PAINT ANALYSIS.

The committee recommends:

(1) A further investigation of the desirability of undertaking:

(a) A collaborative study of methods of chemical and physical examination of paint products and mixed paints, including oils and varnish.

(b) A study of paint legislation with the view to fostering uniformity in laws, definitions, and regulations.

(2) That a convocation of administrative officers and chemists engaged in paint control work be called at the next meeting of the association for the purpose of beginning a systematic study of the problem and for the development of a plan of procedure if it should be decided to be desirable for the association to undertake this work.

W. F. HAND,	W. T. PEARCE.
J. W. KELLOGG,	

*Committee to Consider the Advisability of Studying  
Methods for the Analysis of Paint.*

Approved.

#### REPORT OF COMMITTEE ON BIBLIOGRAPHY.

The Committee on Bibliography for the *Book of Methods*, which was appointed by the president last year, consists of G. S. Fraps, H. D. Haskins, F. P. Veitch, W. W. Randall, and the secretary of the association. There has been considerable correspondence, and much thought

has been given to the subject, especially because of the revision of the *Book of Methods* to be published very shortly.

The committee is of the opinion that the term "bibliography" is not properly used in the present volume of the *Book of Methods*, and that the references cited in the book should, more properly, be referred to as "selected references". A complete list of references, such as is contemplated in an ordinary bibliography, would, in the opinion of the committee, be entirely out of place in the *Book of Methods*, yet a complete bibliography is of exceeding value to the student who desires to undertake a critical review of the development of any of our official methods. The committee is of the opinion that in addition to a bibliography there should be prepared from time to time a critical review of the subjects that are considered in the several chapters of the *Book of Methods*. These reviews, with the bibliography, should be made by someone who has been identified with the referee work of the association and who has acquired a large and comprehensive knowledge first hand, not only of the work of the association, but of the development of the methods by its scheme of collaborative work. With this thought in mind, the committee would like to recommend that there be undertaken an organized plan for creating a comprehensive bibliography and review of our methods. The idea is to appoint for each series of projects, as represented by the chapters in the *Book of Methods*, a reviewer, perhaps designated a special referee, whose duty it shall be to bring down to date the literature and references pertaining to a particular subject, with a critical review. These reviews should be made periodically, coincident, perhaps, with the reprinting of the *Book of Methods*. Such reviews, with the complete references, might properly appear in *The Journal* of the association, and thereby they would be available as a permanent record to any one desiring to make a critical study of a particular method.

It will be remembered that several years ago R. N. Brackett prepared such a critical review of the work done in the study of the method for the determination of phosphoric acid. This work proved to be of tremendous value. The committee realizes that if this undertaking is to be successful, exceptional care must be exercised in the selection of the person or persons to make the reviews, and that owing to the immense amount of work involved in the study of the past work of the association considerable time must elapse before such a plan can be put into successful operation. Once, however, the survey is made and brought down to date, it should be maintained with comparatively little effort.

W. W. SKINNER,  
G. S. FRAPS,  
F. P. VEITCH,

H. D. HASKINS,  
W. W. RANDALL.

*Committee on Bibliography.*

It was moved, seconded, and carried that the report be accepted, and that the committee be continued with authority to proceed with the preparation of such reports as it may deem advisable.

### REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of R. W. Balcom, Chairman of the Board of Editors, covering the period from November 1, 1923, to September 30, 1924, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from November 1, 1923, to September 30, 1924, and found the same to be correct as reported.

H. H. HANSON,  
J. H. MITCHELL.

*Auditing Committee.*

Approved.

### REPORT OF NOMINATING COMMITTEE.

The nominating committee desires to place in nomination the following names:

*President:* C. A. Browne, Washington, D. C.

*Vice-President:* H. D. Haskins, Amherst, Mass.

*Secretary-Treasurer:* W. W. Skinner, Washington, D. C.

*Additional members of the executive committee:*

W. W. Randall, Baltimore, Md.

W. H. MacIntire, Knoxville, Tenn.

A. J. PATTEN,      W. F. HAND.  
G. W. HOOVER,

*Nominating Committee.*

It was moved, seconded, and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

### REPORT OF COMMITTEE ON RESOLUTIONS.

Since the last meeting the association has lost by death four of its members: Prof. W. A. Withers, Dr. W. C. Stubbs, R. W. Hilts, and A. W. Ogden.

Prof. William Alphonso Withers was active as a teacher and as an investigator, and in many other lines of public work. In 1910 he served as president of this association. As a teacher he was particularly suc-

cessful, as a number of members of this association, who were his former pupils, testify.

The committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Prof. W. A. Withers this association has lost a former officer and a member who greatly aided the cause of agricultural chemistry by his unselfish service and devotion to his work, while those who had the privilege of knowing him have lost a true and loyal friend.

Dr. William Carter Stubbs, formerly Director of the Louisiana Agricultural Experiment Station, died at New Orleans, La., July 7, 1924.

Dr. Stubbs was Director of the Louisiana Agricultural Experiment Station and Professor of Agriculture at the Louisiana State University from 1885 to 1905. During the early days of this association he was an active member and contributed to its success. As a writer and teacher Dr. Stubbs was considered one of the leaders in his particular field.

As it is fitting that this association place on record its expression of the loss of a sincere friend, the committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Dr. W. C. Stubbs this association has lost an esteemed member and a true friend—one whose influence was felt in the early development of the association; the cause of scientific agriculture has lost an earnest worker; and the students of this subject have lost a great teacher.

Roy Wilson Hilts, of the Bureau of Chemistry, and Chief of the Western Food and Drug Inspection District, died January 12, 1924.

The committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Mr. R. W. Hilts this association has lost a valued member—one who has rendered efficient service—and many of the members have lost a warm personal friend.

Alfred W. Ogden, for many years a member of this association, died January 21, 1924.

The committee recommends the adoption of the following resolution:

*Resolved*, That this association deplores the loss of its former member, Mr. A. W. Ogden, in whose passing science has lost a valuable worker.

*Resolved*, That these resolutions be printed in the proceedings of this association, and that copies be sent to the families of our deceased friends and coworkers.

*Resolved*, That this association express its regret that owing to illness the Honorable Henry C. Wallace<sup>1</sup> was unable to attend this meeting and tender its best wishes for his rapid recovery.

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<sup>1</sup> Died October 25, 1924.

*Resolved*, That this association express to its honorary president, Harvey W. Wiley, its congratulations on his 80th birthday and its heartiest wishes for many more years of useful service.

*Resolved*, That this association desires to express its appreciation of the able and efficient manner in which the president, R. E. Doolittle, has discharged the duties of his office.

*Resolved*, That this association wishes to commend the Chairman of the Board of Editors, R. W. Balcom, and his coworkers and assistants for their efficient and able services in conducting the affairs of *The Journal*.

*Resolved*, That this association wishes to commend the efficient work of the secretary, W. W. Skinner, and his assistants, especially Miss Marian E. Lapp, for their untiring and highly successful efforts in making the convention a success.

*Resolved*, That this committee desires to express its highest appreciation of the able, diligent, and earnest work of the Chairman of the Committee on Editing Methods of Analysis, as well as the efforts of his coworkers, especially in the revision of the *Book of Methods*.

*Resolved*, That the thanks of the association are due the management of the Raleigh Hotel for the use of various rooms and for other courtesies extended to the association.

G. S. FRAPS,

A. P. KERR.

W. M. ALLEN,

*Committee on Resolutions.*

Approved.

## CONTRIBUTED PAPERS.

### PRELIMINARY NOTES ON THE DIRECT DETERMINATION OF MOISTURE<sup>1</sup>.

By GEORGE L. BIDWELL and WILBUR F. STERLING (Bureau of Chemistry, Washington, D. C.).

#### INTRODUCTION.

Moisture is ordinarily considered as being held in organic materials in much the same manner as water is held in a wet sponge. The removal of moisture from such materials has occasioned much difficulty in the past. Drying in an oven at times sets free water held by surface phenomena, water of crystallization, and water of constitution; it may drive off water that is the result of chemical reactions involving extreme changes in constitution.

The results obtained by oven drying depend upon the completeness of the removal of this water. Inherent errors in the method of oven drying are the result of oxidation during heating, loss by volatilization of substances other than water, and sealing in of water by varnish-like films. The lack of homogeneity in a sample also introduces serious errors in any moisture determination, but this factor can be overcome to some extent by using relatively large samples.

The ideal moisture method, which will probably never be found, would be applicable to all substances, would be rapid, would require little skill, would separate and determine uncombined water and nothing else, and, preferably, would determine water directly and not by difference. The method here submitted does not have all these advantages, but for many products it offers an improvement over present methods in accuracy and speed.

#### HISTORICAL.

Marcusson<sup>2</sup> was the first to determine moisture by distilling the sample with a liquid immiscible with water. The sample was introduced into a flask containing a measured quantity of xylene, and the mixture was distilled into a receiver constricted at the bottom to a calibrated tube until several hundred cubic centimeters passed over. The column of water was then measured. This method with variations has been used with varying degrees of success on a variety of materials.

Rogers<sup>3</sup> proposed using toluene to determine the moisture of leather, and the process was essentially the same as that used by Marcusson.

Dean and Stark<sup>4</sup>, whose article includes a very comprehensive bibliography, devised an apparatus whereby the sample was refluxed with

<sup>1</sup> Read at the Fortieth Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., October 20, 1924.

<sup>2</sup> *Mitt. k. Materialprüfungsamt*, 1905, 23: 58

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 137, p. 172

<sup>4</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

a liquid immiscible with water, the receiver being essentially a calibrated sedimentation tube with a side arm, which returns the medium to the flask, while the water is trapped by dropping to the bottom of the calibrated tube. While this tube is well adapted to the purposes for which it was devised it was found to be insufficiently accurate for determining small quantities of moisture. Several modifications of this device have also been proposed.

The method discussed in this paper resulted from an attempt to modify and correlate Dean and Stark's apparatus and methods and to find a liquid that would distil over all the water without decomposing the sample and liberating chemically formed water. The use of the principle involved was extended to make the method applicable to a wide variety of materials, especially those in which it is difficult to determine moisture by the conventional drying methods.

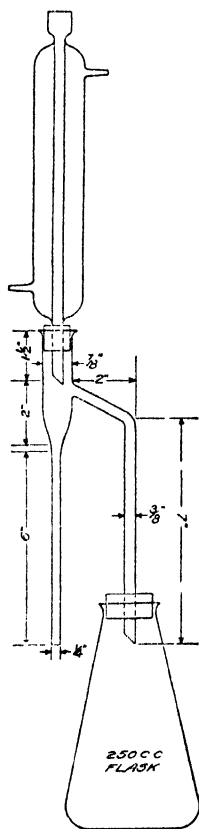


FIG. 1. DIAGRAM OF THE APPARATUS USED.

#### METHOD.

#### APPARATUS.

The tube proposed by the authors of this paper is identical in principle with the apparatus used by Dean and Stark to determine the water in petroleum emulsions and similar products. It has been adopted for several products high in moisture and in those cases where extreme accuracy is not necessary. By reducing the diameter of the calibrated receiving tube it has been found that the column of water can be read with sufficient accuracy for a wide variety of products.

The diameter of the receiving tube used by Dean and Stark was found to be too large to read accurately so as to obtain good duplicates or comparable results. A calibrated section taken from a 5 cc. Mohr pipet, sealed at one end and attached to the apparatus as shown in the cut, entirely overcomes this difficulty.

Most of this work has been done with a free flame, although it is advisable to use a bath or hot plate, especially for samples containing sugar.

#### DETERMINATION.

Introduce into a 250 cc. Pyrex Erlenmeyer flask sufficient sample to give from 2-5 cc. of water. If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely, usually about 75 cc., and connect the apparatus

as shown in Fig. 1. Fill the receiving tube with toluene by pouring through the top of the condenser. Bring to a boil and distil slowly, about two drops per second, until most of the water has passed over; then increase the rate of distillation to about four drops per second. When the water is apparently all over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distil over; if it does, repeat the washing down process. If any water remains in the condenser, remove it by brushing down with a tube brush attached to a copper wire and saturated with toluene, washing down the condenser at the same time. The entire process is usually completed within an hour. Allow the receiving tube to come to room temperature. If any drops adhere to the sides of the tube they can be forced down by a rubber band wrapped around a copper wire. Read the volume of water and calculate to percentage. The tube is calibrated in tenths of a cubic centimeter, and the column can be read to hundredths with reasonable accuracy.

It is necessary to have the condenser and receiving tube chemically clean in order to prevent an undue quantity of water sticking to the condenser and drops of water adhering to the sides of the receiving tube. Clean with chromic sulfuric acid, rinse with alcohol, and dry in an oven.

#### EXPERIMENTAL.

In order to determine to what extent water could be recovered by distilling with toluene 1 cc. of water was added to about 75 cc. of dry toluene and run in the usual manner. The receiving tube contained 0.98 cc. of water. The apparatus was allowed to cool, and another cubic centimeter of water was added with a reading of 1.98 cc. of water. This process repeated twice resulted in readings of 2.98 cc. and 3.98 cc., respectively. Two additional experiments were made, and the same values were obtained. In this way the apparatus is standardized. The tube in question is read with a +0.02 cc. correction.

Ten grams of cupric sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) was run, and 2.90 cc. of water was recovered. This compares well with the theoretical quantity of 2.89 cc. for 4 molecules of water of crystallization. A similar experiment, using 10 grams of sodium sulfate ( $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ ) gave 1.67 cc. of water. The theoretical quantity for 3 molecules of water is 1.68 cc.

Unpublished work on certain materials, especially dried fruits, indicated that distilling with xylene gave higher results than those obtained by the usual drying methods. Xylene boils at  $139^\circ\text{C}.$ , and this high temperature was assumed to break down sugars and other easily decomposable substances which set free water formed in the chemical reactions during decomposition.



The following experiment shows that toluene does not and xylene does decompose levulose with the formation of water.

A sample of impure levulose was desiccated over sulfuric acid for three weeks, ground, and sieved. Four 10 gram samples were weighed out and to two of them 1 cc. of water was added; all the samples were distilled.

10 grams of levulose gave.....	0.37 cc. H <sub>2</sub> O
	0.36 cc. H <sub>2</sub> O
10 grams of levulose plus 1 cc. of water gave....	1.35 cc. H <sub>2</sub> O
	1.35 cc. H <sub>2</sub> O

These residues were not charred.

To another 10 gram sample 1 cc. of water was added, and the determination was carried out with xylene instead of toluene. The receiving tube contained 2.04 cc. of water. The residue was dark brown.

This indicates that the xylene boiling at a higher temperature broke up the levulose with the production of water. Toluene, which boils at 110°C., was substituted, therefore, and it was found to eliminate this undesirable feature in nearly all cases.

The method has been tried on enough samples to show its wide range of usefulness, as shown in Table 1. Those samples that require special consideration will be briefly mentioned.

*Raisins.*—No difficulties were experienced in the manipulation. The residue consisted of hard, dry lumps. Duplicate determinations checked very well.

*Flour.*—The samples bumped badly. This difficulty was practically overcome by the addition of sufficient dry sand to cover the bottom of the distilling flask. Comparable results with the oven method were obtained.

*Butter.*—The fats dissolved in the solvent, and the curd and salt settled to the bottom of the flask. The determination was completed in a half hour. The results compared favorably with those obtained by oven drying.

*Dried Milk.*—No difficulties were encountered during the determination. Duplicate determinations gave excellent checks.

*Leather.*—The mixture boiled smoothly. Results checked well with those obtained by an oven method months previously.

*Molasses.*—At first the samples were on the bottom of the flask. As boiling proceeded part of the molasses floated on top and stuck to the sides of the flask. The residues were brown, crumbly masses. The method compared well with the oven method on these samples.

*Honey.*—During the determination the sample remained on the bottom of the flask. There was considerable darkening. However, the results compared well with those obtained by oven drying. The residue was a brown, homogeneous, semi-solid mass.

*Karo sirup.*—The action was similar to that of honey. Much less discoloration was observed.

*Strawberry jam.*—A large lump was used as a sample. Three hours was required to get complete dehydration. The residue was a hard mass. It is evident that materials of this sort should be spread out so as to expose more surface.

*Dried apples.*—The samples gave no difficulty whatever during the determination. The residues were hard granules of about the same color as the original samples. The duplicate determinations checked very well.

*Eggs.*—In determining moisture in fresh eggs it was observed that the sample was not sufficiently homogeneous for accurate checks. There was considerable difficulty occasioned by water sticking in the condenser and adhering to the sides of the tube. Duplicate determinations agreed fairly well considering the nature of the sample. A further study of eggs and other substances of a similar nature will be made.

*Green grass.*—The sample gave no difficulty in manipulation. The residue was bright green and crumbly. Duplicate determinations gave reasonable checks for such a heterogeneous sample.

*Peat.*—Unground samples gave as close agreement between duplicates as could be expected.

#### DISCUSSION.

##### *Disadvantages.*

(1) The tubes require thorough cleansing before each determination, and the condenser should be cleaned once for every two or three determinations. Otherwise it is necessary to brush down the condenser so that the water will flow to the bottom of the tube as it should.

(2) Water of crystallization is separated from some substances as copper sulfate, sodium sulfate, etc.

(3) Several substances, such as alcohol, glycerin, acetone, etc., which are volatile and miscible with water, may distil over and cause high results by this method.

##### *Advantages.*

(1) The method determines water directly, and the results are actual water and not loss in weight.

(2) As the substances mentioned above, which are volatile and miscible with water, are more or less soluble in toluene, the results by this new method are more accurate than results given by an oven method.

(3) The results are obtained in most cases within an hour, and in all cases in much less time than a working day.

(4) No complicated expensive apparatus is required. The tubes can be made by any glass blower and should be comparatively inexpensive if manufactured in quantity. The other parts of the apparatus are found in every laboratory.

TABLE 1.  
Comparison of new method with oven methods\*.

SAMPLE	MOISTURE			
	Toluene Method		Oven Methods	
	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>	<i>average per cent</i>
Gray shorts.....	12.45		12.42	
Patent flour.....	12.60		12.68	
	12.70	12.65	12.81	12.75
Patent flour.....	12.80		12.89	
	12.85	12.83	12.94	12.92
Flour.....	11.45			
	11.40	11.43	11.46	
Flour.....	13.85		13.82	
	13.80	13.83	13.81	13.82
Butter.....	13.76			
	13.77	13.77	13.84	
Oleomargarine.....	11.38			
Oleomargarine... ..	11.23			
Raisins.....	13.1		13.08	
	13.1	13.10	13.09	13.09
Raisins.....	15.0		13.13	
	14.9	15.0	13.20	13.17
Raisins.....	15.4		13.57	
	15.4	15.4	13.52	13.55
Raisins.....	13.3		11.06	
	13.3	13.3	11.05	11.06
Eggs... .. Max. ...	76.91		72.40	
Min....	72.79	74.70†	72.43	72.42
Beet molasses.....	21.90		21.92	
Cane molasses.....	20.42		20.34	
Honey ... ..	19.60		19.33	
	19.57	19.59	19.47	19.40
Leather ... ..	11.60		11.15	
Leather.....	5.73		5.60	
Dried milk.....	8.15			
	8.10	8.13		
Dried milk.....	6.53			
	6.53	6.53		
Strawberry jam... ..	31.10			

\* All oven results except those on gray shorts and eggs were determined by analysts working entirely independently of the authors. The oven methods used were those ordinarily employed in the routine determination of moisture in the various materials.

† Average of seven determinations.

TABLE 1.—Continued.

SAMPLE	MOISTURE			
	Toluene Method		Oven Methods	
	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>	<i>average per cent</i>
Fresh grass . . . . .	86.50 84.50	85.50		
Peat . . . . .	81.40 81.35 78.46	80.40		
Karo sirup . . . . .	20.3			
Dried apples . . . . .	14.00 13.97	13.99	12.26 12.41	12.34
Ground wheat . . . . .	11.1		11.18	
Ground wheat. . . . .	11.5		11.42	
Ground wheat . . . . .	13.6		13.56	

(5) The effect of humidity during the determination is eliminated.

(6) The method prevents oxidation of the sample while the moisture is being determined.

(7) No special training in technique is required for the manipulation of the method as several analysts unacquainted with it were able, after simple explanations, to obtain entirely satisfactory results at the start.

In future work the authors intend to make a thorough study of a large variety of materials and determine the limits of usefulness of the method. Certain ideas concerning further modifications of the apparatus will be tried, with the view to reducing the technical disadvantages to a minimum.

## THE QUANTITATIVE DETERMINATION OF MOISTURE IN WHEAT FLOUR<sup>1</sup>.

By G. C. SPENCER (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

### INTRODUCTION.

Wheat flour consists of extremely fine particles of an organized body; they vary in cellular structure and chemical nature and offer an enormous surface for attracting and retaining atmospheric moisture. This fact has not been given due consideration by previous workers in this field and, unfortunately, wheat flour has been regarded in the same light as any other finely divided solid.

<sup>1</sup> Read at the Fortieth Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., October 21, 1924.

For determining moisture in flour chemists have usually dried known quantities of flour, each using his own method and whatever drying apparatus was most convenient, and reported the *loss in weight* as moisture.

The present food standards specify that flour should contain "not more than thirteen and one-half per cent (13.5%) of moisture". This is the standard that was recommended by a committee of the Association of Official Agricultural Chemists to the Secretary of Agriculture and adopted and proclaimed by him in December, 1904, as "the official standard of purity for this product for the United States of America".

For a detailed review of the establishment of 13.5 per cent as the legal limit for moisture in flour, the reader is referred to the excellent pamphlet on the subject by Harry Snyder<sup>1</sup>.

#### DISCUSSION.

The experience of all investigators points to the fact that all the moisture can not be removed from flour by any process of mere drying. The same statement may be made concerning pulverized coal and many other cellular materials. The moisture present in these finely powdered substances seems to be so held that the water particles most intimately in contact with the solid particles of flour, or other cellular tissue, adhere more tenaciously than that portion of the water which is farther removed from the solid surfaces. If one applies the simple law of attraction between two bodies, the influence of proximity between the moisture and flour particles would seem to account for the inadequacy of simple drying to remove the last of the water, since the vapor pressure is insufficient to overcome the adsorption.

The best expression of moisture values for flour, therefore, may be stated as the percentage loss in weight of a flour sample dried under conditions that may be prescribed by a *convention of all interested parties*.

These statements seem to be justified by the accurate moisture measurements of O. A. Nelson and G. A. Hulett<sup>2</sup> on samples of wheat flour and other powdered colloidal materials. Their work has further indicated the improbability of the theory that water of hydration exists to any appreciable extent in flour particles. Reference to the table of results and to Fig. 3 in the original publication of these authors will show that no break occurs in the moisture percentage curve for wheat flour up to 184°C., after which decomposition of the flour proceeds rapidly.

The possibility of more or less oxidation of flour by drying in air has been refuted by the work of F. T. Shutt and P. J. Moloney<sup>3</sup>.

<sup>1</sup> Wheat Flour, Its Weight and Moisture Content, 2nd ed., 1923. Published by the Millers' National Federation, Chicago, Ill.

<sup>2</sup> J. Ind. Eng. Chem., 1920, 11: 40

<sup>3</sup> Trans. Roy. Soc. Canada, Series III, 1917, 11: 101.

The Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists<sup>1</sup> gives the following directions for the estimation of moisture in flour:

Dry a quantity of the substance, representing about 2 grams of dry material, in a current of dry hydrogen, or in vacuo at the temperature of boiling water to constant weight (approximately 5 hours).

The official method fails to specify the partial pressure to be used. It is not strange, therefore, that different chemists, using whatever partial pressure was available in their several laboratories, should report such widely varying results on the same lots of flour as are found in the literature in general. This deplorable lack of uniformity has led to great confusion and misunderstanding among control chemists, and it is the purpose of the writer to suggest conditions of drying flour that may serve as a basis for a satisfactory revised method.

#### PURPOSE OF INVESTIGATION.

In the spring of 1923, the writer of this paper commenced a series of experimental determinations of moisture in flour. Three representative types of flour were procured, *viz.*, hard wheat patent, hard wheat clear, and soft wheat straight. Sufficient quantities of these samples to last throughout the entire work were placed at once in wide-mouth, glass-stoppered bottles. The samples were thoroughly mixed every time fresh charges were taken for test, and it is believed that a uniform moisture content throughout each of the samples was maintained.

The experimental work was conducted along four separate lines, as follows:

1. A trial of methods that have hitherto been used in the Bureau of Chemistry for the drying of flours.
2. A comparison of results obtained by drying at reduced pressures.
3. The development of a vacuum method to be used as an umpire method.
4. The development of a rapid method to be used as a routine method.

#### EXPERIMENTAL WORK.

In order to avoid repetition, certain conditions common to all the determinations will be stated, unless otherwise noted, in the following paragraphs:

*Weights of samples.*—Samples of approximately 2 grams (catch weight) were used. After drying they were cooled in a desiccator about 20 minutes and then weighed.

*Containers.*—Aluminum dishes with fitted inside covers, 18 mm. deep and 60 mm. in diameter, were used; the average weight was about 9.3 grams. All the dishes were kept tightly closed except while drying in the oven.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 71.

*Vacuum oven.*—The vacuum oven was cylindrical, water-jacketed, and heated by gas flames. The drying chamber measured 18 inches in length by 8 inches in diameter.

*Electric oven.*—The oven was of a standard make now in common use, and was provided with a thermostat that permitted accurate temperature adjustment.

*Desiccating agents.*—Calcium carbide and reignited calcium oxide were used in the desiccators. Sulfuric acid was tried at first, but as it showed no advantage over the more convenient oxide or carbide of calcium its use was discontinued after a few weeks.

*Vacuum pump.*—The lowest working pressure attained in these experiments was 5 millimeters of mercury. This pressure and other pressures were satisfactorily maintained by use of a small motor-driven pump which exhausted into the house vacuum system. Any desired pressure could be obtained by allowing air to enter the vacuum line through a control valve in a side tube between the manometer and the pump, the oven, of course, being at the end of the line.

*Partial pressure readings.*—The partial pressure readings are expressed in millimeters or inches of mercury.

*Temperatures.*—The temperatures are stated in centigrade degrees.

*Graphs*<sup>1</sup>.—In most cases the results in this paper are expressed by graphs which give the percentages of moisture found. The relative humidities for the corresponding days, taken at the time of weighing the charges, are shown by a dotted line. These graphs are presented in lieu of the more cumbersome tabulations.

As a rule two samples each of the three flour types (six in all) were heated in the drying oven daily under like conditions.

## 1. PREVIOUS METHODS.

The older methods were included in this experimental work partly as a matter of record and partly to determine by a multiplicity of tests on the same samples whether or not any one of these methods will give closely agreeing results from day to day under changing conditions of atmospheric humidity. The results, shown in Graphs 1 to 4, are surprisingly irregular on different days although, as a rule, the duplicates for any one day are fairly close. The variations noted may be due largely to changes in the relative humidity of the atmosphere.

In making determinations for moisture in flour, using a current of hydrogen for 5 hour periods at the temperature of boiling water, the two following procedures were used:

*Method A (Caldwell*<sup>2</sup>*).*—The flour charge was weighed into an open glass tube containing an asbestos mat on a perforated disk, which was

<sup>1</sup> The graphs were drawn by H. C. Hunter, of the Bureau of Chemistry.

<sup>2</sup> H. W. Wiley. Principles and Practices of Agricultural Chemistry, 1914, 3: 29.

supported by a crimp in the lower end of the tube. This tube was also fitted with a ground-glass stopper in order to protect the flour from the air when the tube was not attached in the drying apparatus. During the drying operation the glass tube was connected by a rubber stopper in an inclined metal tube, which in turn was surrounded by steam. The hydrogen gas, dried and previously heated, entered the lower end of the metal tube and passed through the perforated disk and the flour charge before escaping through a small sulfuric acid trap attached to the upper end of the tube.

*Method B.*—In the other type of hydrogen drying apparatus the glass tube described under Method A was hung vertically from a rubber stopper in a larger tube which was surrounded by steam. The hydrogen in this case entered at the top of the tube and passed through the flour and asbestos mat from above; it was then led into the air through a small trap similar to that described in Method A.

TABLE 1.  
*Moisture results obtained by drying in hydrogen.*  
(Temperature, 98°–100°C ; time, 5 hours )

SAMPLE	METHOD A				METHOD B			
	No of tests	Maximum	Minimum	Average	No of tests	Maximum	Minimum	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Patent flour	4	12.47	6.51	10.29	8	12.59	8.37	10.58
Clear flour	1	..	..	11.16	9	11.71	6.82	9.72
Straight flour...					7	13.21	8.88	11.64
Corn grits..	2	9.34	9.13	9.24			..	..
Linseed meal	5	8.22	7.88	8.05				
Middlings	1		..	8.82				
Cottonseed cake	1			7.28	1			6.61
Apple pomace					2	6.49	6.02	6.25

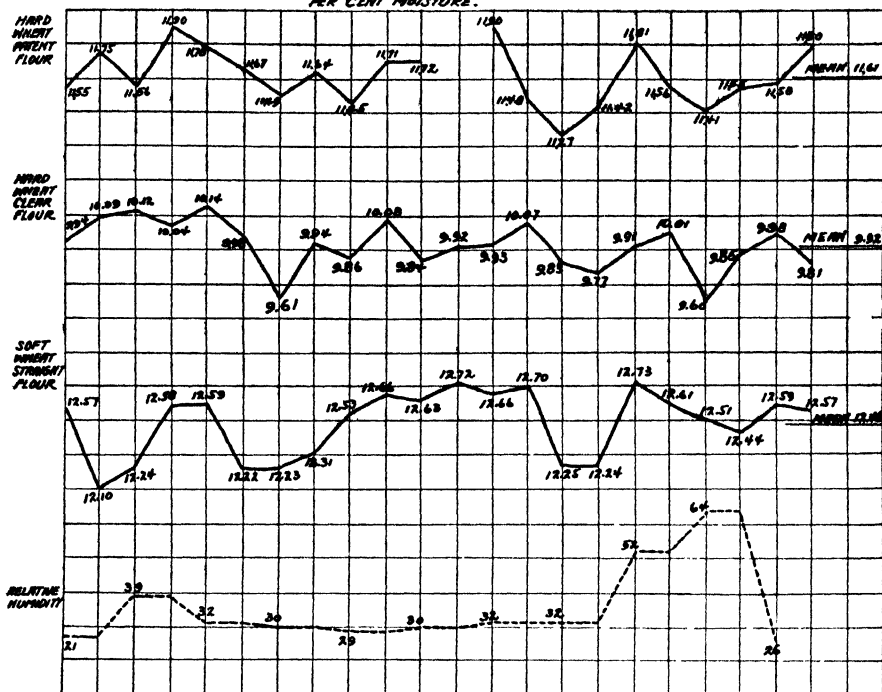
The flour moisture results obtained by both of these methods were unsatisfactory, as shown in Table 1. The moisture already present in the flour seemed to act on the proteins, causing the formation of a dough-like mass which prevented enough of the moisture from being carried out by the stream of gas to cause irregularities in the percentages indicated. In no case were the results comparable with those obtained by other drying methods in point of reliability, and the duplicate determinations rarely agreed. On the other hand, when either hydrogen method was applied to coarser materials, like corn grits or linseed meal, the results were fairly concordant.

## 2. COMPARATIVE FLOUR-DRYING EXPERIMENTS AT REDUCED PRESSURES.

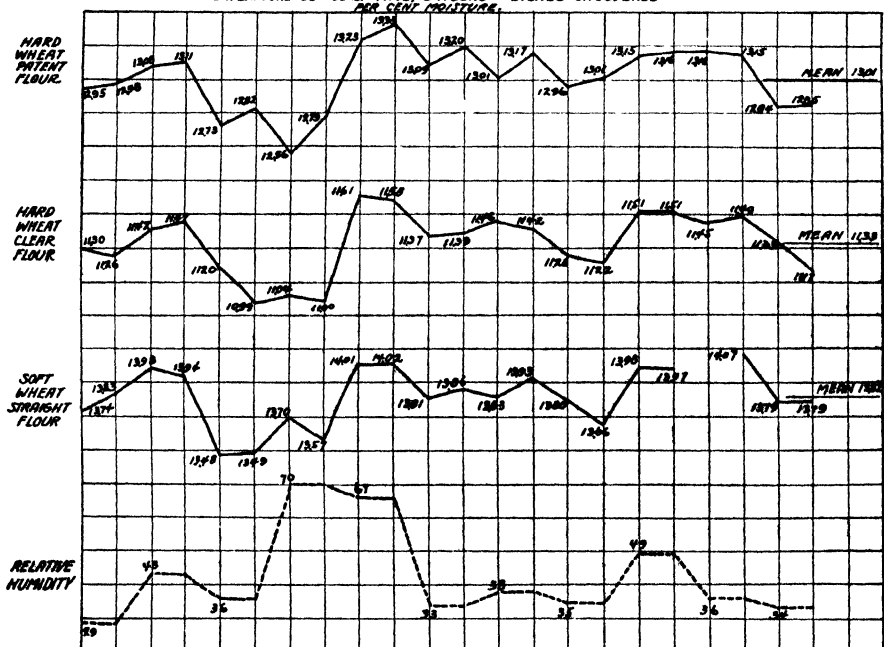
In the experimental work, which subsequently led to the results shown in the graphs, many trials were made by drying various weights



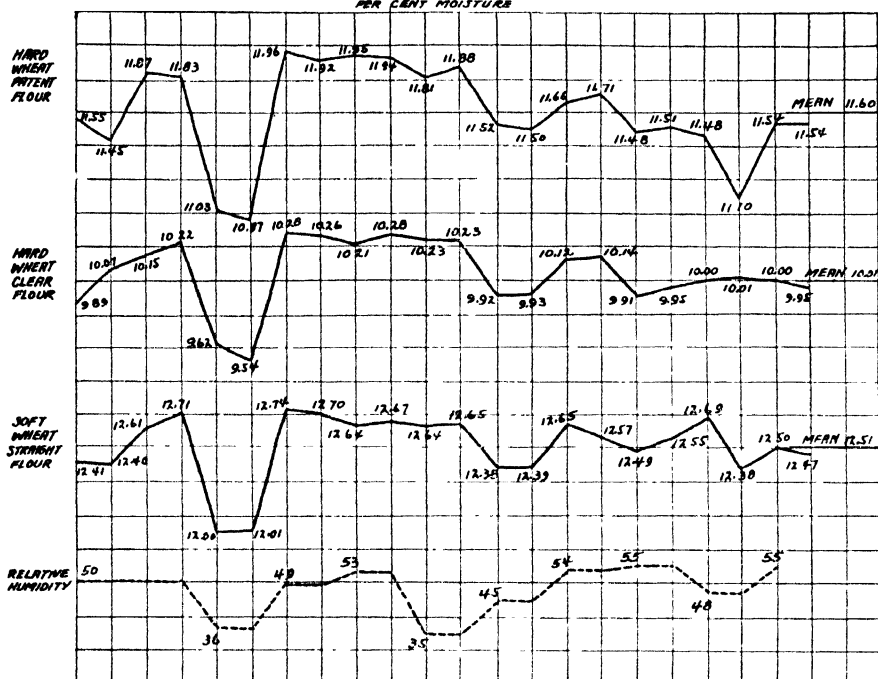
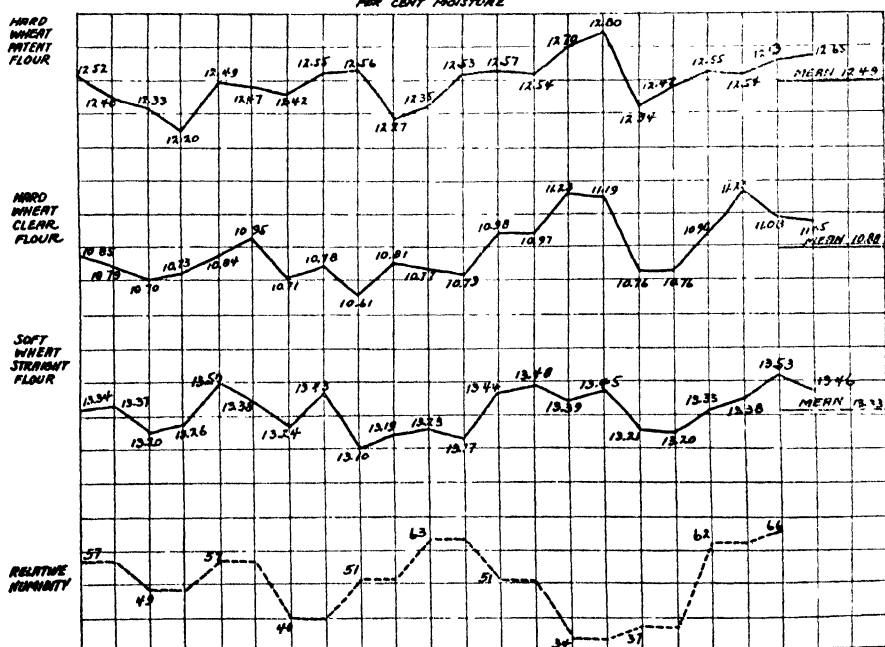
**1. WATER OVEN METHOD.**  
 TEMPERATURE 30-100°C. TIME, 5 HOURS. DISHES UNCOVERED.  
 PER CENT MOISTURE.



**2. VACUUM OVEN METHOD - 127 TO 203 MM. PRESSURE.**  
 TEMPERATURE 30-100°C. TIME, 5 HOURS. DISHES UNCOVERED.  
 PER CENT MOISTURE.



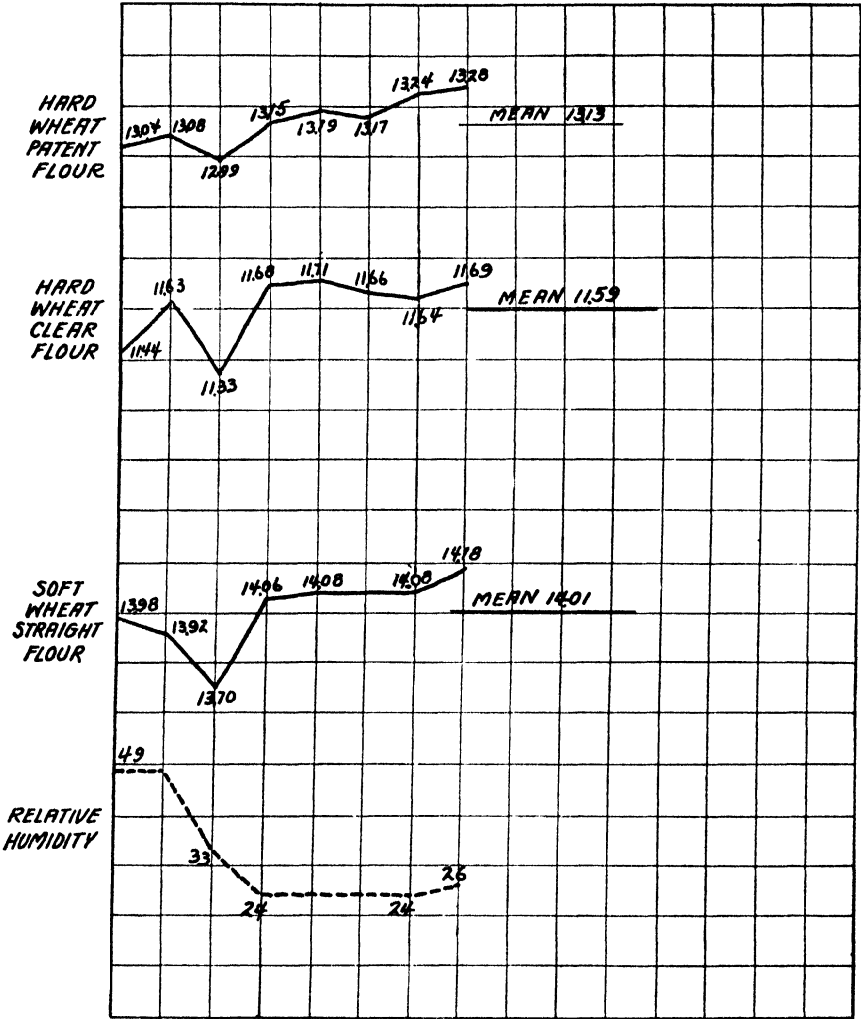
## 3. VACUUM OVEN METHOD AT 70°- 127 TO 203 MM. PRESSURE

TIME 5 HOURS. DISHES UNCOVERED.  
PER CENT MOISTURE4. ELECTRIC OVEN  
TEMPERATURE 111°C. TIME 5 HOURS DISHES UNCOVERED  
PER CENT MOISTURE

of flour under different conditions of pressure and for different periods of time. The conclusions drawn from these experimental tests are the basis of the recommendations that will follow.

5. VACUUM OVEN METHOD. OPEN DISH.

TEMPERATURE 98-100°C. PRESSURE 25 MM. TIME, 5 HOURS.  
PER CENT MOISTURE.



It may be of interest at this time, however, to record the moisture results that were obtained at pressures of one, two, three, and four inches of mercury in a vacuum water oven, since these demonstrate the necessity of controlling the pressure within the oven as well as the other conditions of drying.

TABLE 2.  
*Comparative moisture results with different partial pressures.*  
 (Temperature, 98°-100°C ; time, 5 hours; dishes loosely covered )

SAMPLES	PRESSURE, 1 INCH				PRESSURE, 2 INCHES			
	No. of tests	Maximum	Minimum	Average	No. of tests	Maximum	Minimum	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Patent flour	12	13.32	13.19	13.25	11	13.20	13.03	13.15
Clear flour	13	11.72	11.60	11.66	11	11.64	11.43	11.58
Straight flour	13	14.13	14.03	14.08	11	14.04	13.87	13.99

	PRESSURE, 3 INCHES				PRESSURE, 4 INCHES			
	No. of tests	Maximum	Minimum	Average	No. of tests	Maximum	Minimum	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Patent flour	11	13.12	12.92	13.03	5	13.13	12.85	12.97
Clear flour	11	11.54	11.39	11.47	5	11.44	11.31	11.39
Straight flour	11	13.95	13.80	13.89	5	13.85	13.65	13.79

### 3. PROPOSED STANDARD VACUUM METHOD (UMPIRE).

The writer has repeatedly used the following method of drying with different types of flour, and the results obtained leave no doubt as to its dependability.

#### *Umpire Method.*

Weigh accurately about 2 grams of the sample in a tared, covered dish. Loosen the cover and heat the dish and contents in a vacuum oven to 98°-100°C. for 5 hours at a pressure of not more than 25 mm. (1 inch) of mercury. Tighten the cover on the dish and cool for 20 minutes in a desiccator. Weigh and calculate the loss in weight as moisture.

Graphs 5 and 6 illustrate the relative advantages of covered and uncovered dishes for drying in vacuum<sup>1</sup>.

It is not claimed that these results show the actual amount of moisture present, but it has been demonstrated that a closer approximation has been reached than by any other practicable method. It is believed that this standard or umpire method, if adopted, might settle many of the misunderstandings and disagreements that often arise in the cereal trade.

### 4. PROPOSED ROUTINE METHOD.

The obvious inconveniences that attend the use of vacuum ovens, as well as the time consumed, led the writer to seek a method that would give the same results as those obtained by careful vacuum drying without the employment of complicated apparatus and needless expenditure of time.

<sup>1</sup> L. C. Mitchell and Samuel Alfend. J. Assoc. Official Agr. Chemists, 1924, 8: 76.

*Routine Method.*

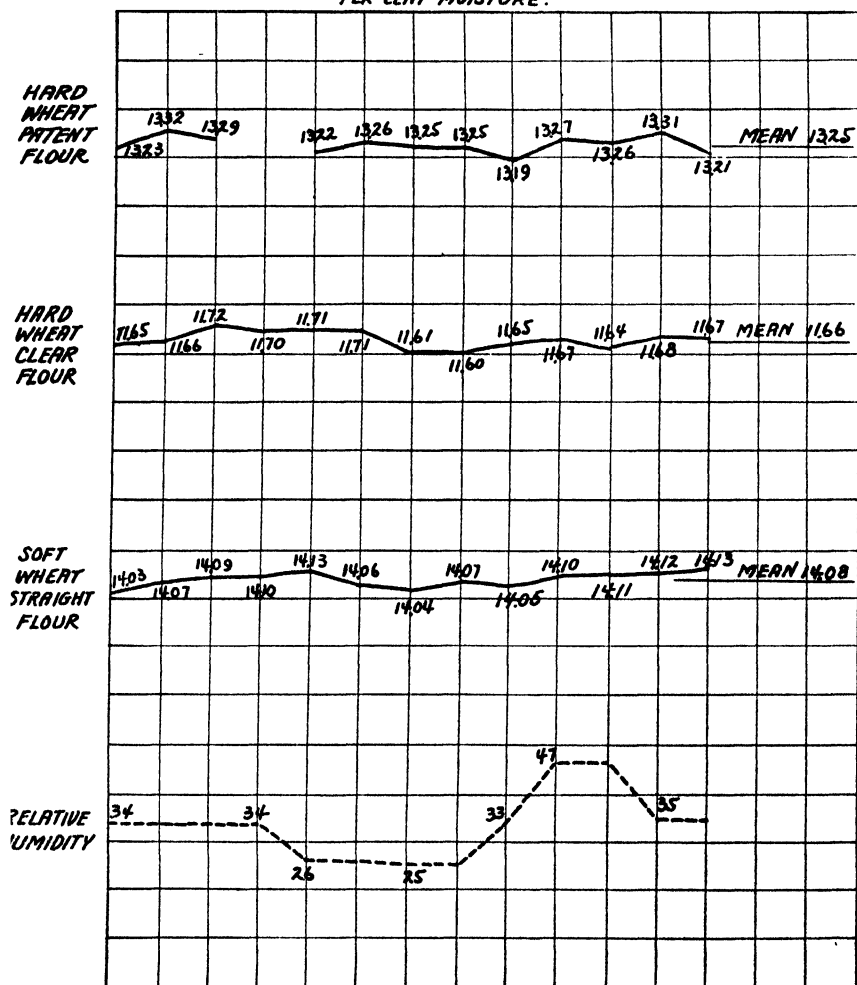
Weigh accurately about 2 grams of the sample in a tared, covered dish. Remove the cover and heat the dish and contents in air in an oven at 130°C. for 1 hour. Replace the cover on the dish and cool in a desiccator for 20 minutes. Weigh and calculate the loss in weight as moisture.

Graph 7 shows the close agreement of results obtained by the routine method with those obtained by the vacuum method and presented in Graphs 5 and 6.

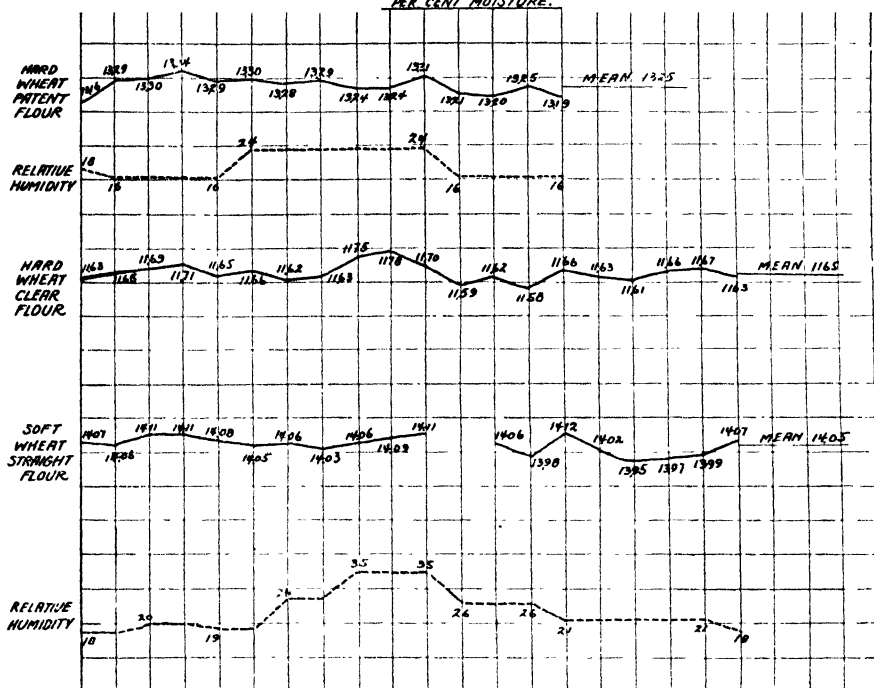
The claims advanced in this paper were carefully verified by six chemists working independently on samples that were distributed by

**6. PROPOSED UMPIRE METHOD.**

TEMPERATURE 38-100°C PRESSURE 25 MM. TIME, 5 HOURS. DISH LOOSELY COVERED.  
PER CENT MOISTURE.



## 7. PROPOSED ROUTINE METHOD.

TEMPERATURE 150°C ATMOSPHERIC PRESSURE TIME 1 HOUR DISH UNCOVERED.  
PER CENT MOISTURE.

the writer as Associate Referee on Cereal Products of the Association of Official Agricultural Chemists.

The author acknowledges with thanks the suggestions of L. H. Bailey and others in the Bureau of Chemistry.

## SUMMARY.

Extensive tests indicate that the determination of moisture in flour by drying in hydrogen, in an air oven, or in a vacuum oven by the usual procedures is not satisfactory.

The advantage of using loosely covered dishes in vacuum drying is demonstrated.

A thoroughly tested method involving the use of a definite partial vacuum is suggested as a standard.

The new and rapid method proposed for the determination of moisture in flour obtains results that show close agreement with those obtained by the proposed vacuum method, and the time of operation is reduced from 5 hours to 1 hour. The necessary apparatus is easily installed, and the cost of upkeep is low.

NOTE.—Since the completion of the work on which this paper is based, a new method for the determination of moisture in a variety of foods and feeding stuffs has been described by G. L. Bidwell and W. F. Sterling. (See p. 295.) Results by this direct method on four samples of flour compared very closely with those obtained by the rapid and convenient method proposed in this paper.

## AN APPLICATION OF THE HOWARD METHOD TO THE DETECTION OF SPOILAGE IN BERRY PRODUCTS.

By GEORGE H. NEEDHAM and CARL R. FELLERS (Department of Food Preservation, University of Washington, Seattle, Wash.).

## INTRODUCTION.

Since the passage of the United States food and drugs act in 1906, the detection of spoilage in food products has become a very important problem.

One class of prepared foods for which methods and standards are urgently needed is that of fruit products. Jellies, jams, fruit pulps, and canned and preserved fruits may appear perfectly sound when examined superficially, yet they may have been prepared from partially decayed or moldy fruits or berries. The success attained by the Howard microscopic method<sup>1</sup> for ascertaining the relative number of yeasts and spores, and bacteria, and for the detection of mold filaments in tomato products suggested that this method of determining the quality of a food product might be applied to canned fruits and berries.

The published work on the application of the Howard method to berry products is very meager. Bitting<sup>2</sup>, in 1915, published microscopic counts of yeasts and spores, bacteria, and molds in commercial and laboratory samples of the juice from canned blackberries and strawberries, but made no attempt to correlate the figures with the actual conditions of the original berries. He did not make any counts on the drained pulp or pulp and juice together. Schneider<sup>3</sup> gave "ratings" of the number of molds, yeasts and spores, and bacteria permissible in canned berries, jams, and jellies. Inasmuch as no experimental figures are presented these ratings are of questionable value. Stevens and Wilcox<sup>4</sup>, in 1917, listed the fungi that attack strawberries. Among these were *Rhizopus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Mucor*, and *Penicillium*. They mentioned that the growth of *Rhizopus nigricans* was very slow at 10°C. or below. Stevens<sup>5</sup> also stated that *Botrytis* and *Rhizopus* were responsible for the large proportion of rot on strawberries. He concluded that strawberries kept better when picked early in the morning even though they were wet. Working toward definite standards to guide the berry grower and canner, the Northwest Cannery Association<sup>6</sup> has suggested that no soft or moldy berries be accepted, and that after 24 hours none of the berries should show visible mold. Reference to the use of decomposed

<sup>1</sup> Howard and Stephenson. U. S. Dept. Agr. Bull. 581.

<sup>2</sup> U. S. Dept. Agr. Bull. 190, p. 53.

<sup>3</sup> The Microbiology and Microanalysis of Foods, 1920.

<sup>4</sup> U. S. Dept. Agr. Bull. 531.

<sup>5</sup> *Phytopathology*, 1919, 9: 171.

<sup>6</sup> Northwest Cannery Assoc., Portland, Ore., Circ. 149.

stock in jams, canned fruits, and similar products has been made by the U. S. Department of Agriculture<sup>1</sup>, when it is stated that berry products showing the presence of excessive mold will be considered adulterated under the food and drugs act.

A considerable number of objections to the Howard direct microscopic count have been pointed out from time to time, but many of them have been overcome, and it is still of great value.

#### EXPERIMENTAL WORK.

Strawberries and blackberries were used for this work because they were plentiful; other varieties were available in limited quantities only or not at all.

*Selection and storage.*—The berries were purchased in the open market at Seattle from the growers, who gave information as to location and kind of soil and certified as to exact time and day of picking. A portion of the berries was canned immediately; the remainder was stored in a room under atmospheric conditions, and the minimum and maximum temperatures for the entire time of storage were recorded.

*Canning.*—The berries were transferred to a pan and washed with running water for a few seconds. One-half pound flat cans were filled with  $5\frac{1}{8}$  ounces of berries and 6 ounces of water. The containers were sealed, sterilized for 10 minutes in an open water bath at  $212^{\circ}\text{F.}$ , and then rapidly cooled in water. They were stored from one week to five months at room temperature before being opened. No spoilage occurred in any of these cans.

*Preliminary examination.*—

(a) The vacuum or pressure test in inches was taken in order to eliminate possible defective cans.

(b) The cut-out weight of fruit per can was made by allowing the contents to drain on an eight-mesh sieve for one minute.

(c) The specific gravity of the strained juice was determined by means of a hydrometer. In all cases this value kept within the narrow limits of 1.016–1.024.

(d) The contents of each can were examined microscopically.

*Preparation for count.*—The contents of the can were poured on the 8-mesh sieve and allowed to drain for one minute. Then the berries were brushed through a 20-mesh sieve, and the seeds and pulp that would not pass through it were discarded. It was found that the use of a stiff scrubbing brush two inches square, made by cutting a cheap vegetable brush in half, was very good for blackberries but not so good for strawberries. For the latter, a flexible spatula was used.

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<sup>1</sup> Service and Regulatory Announcements No. 27, item 372.



*The count.*—The microscopic count given in Bulletin 581, United States Department of Agriculture, and also in the Methods of Analysis of the Association of Official Agricultural Chemists<sup>1</sup> was used. An attachable binocular eyepiece, fitted with  $\times 6.4$  eyepieces used with an 8 mm. objective, was found by the authors to be ideal for counting yeasts and spores<sup>2</sup>.

*General outline.*—The experiments were divided into three different series, termed I, II, and III, respectively. In Series I, part of the berries that were one day old at purchase was canned immediately; another portion was canned when two days old; another, when three; and finally another, when four days old. The percentage of sound, of slightly soft, of very soft, and of moldy berries by count was noted in each case and recorded. In Series II some of the cans of Series I were opened; one portion was made into jam, another portion was made into jelly, and a small part was reserved as a stock sample. In Series III the berries were carefully sorted, and moldy berries either five or six days old were added to the sound ones in a series of amounts by weight varying from 0–100 per cent.

#### SERIES I.

##### *Berries Canned at Intervals after Picking.*

In this series each can contained  $5\frac{1}{8}$  ounces of berries and 6 ounces of water, and the percentage by count of sound, of slightly soft, of very soft, and of moldy berries was recorded. By "moldy" is meant any berry that showed visible mold, irrespective of whether a small part or the entire berry was affected. Four cans of blackberries one day, two days, three days, and four days old were designated B-1, B-2, B-3, and B-4, respectively, according to age. Identical series for strawberries were marked S-1, S-2, S-3, and S-4. Three lots of both blackberries and strawberries obtained from different sources were utilized.

The results of the counts are shown in Table 1.

#### SERIES II.

##### *Jams and Jellies from Series I.*

*Blackberries.*—Two cans of the B-1 berries were opened, and the contents were mixed. A sufficient quantity of this stock was reserved for a count, and half of the remainder was made into jam and half into jelly. For jam, 55 parts by weight of sugar were added to 45 parts by weight of the mixed berries and juice. For jelly, equal parts by weight of sugar and stock were used. In the case of jelly the pulp was expressed

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 164.

<sup>2</sup> *Science, New Series*, 1924, 59, 341.

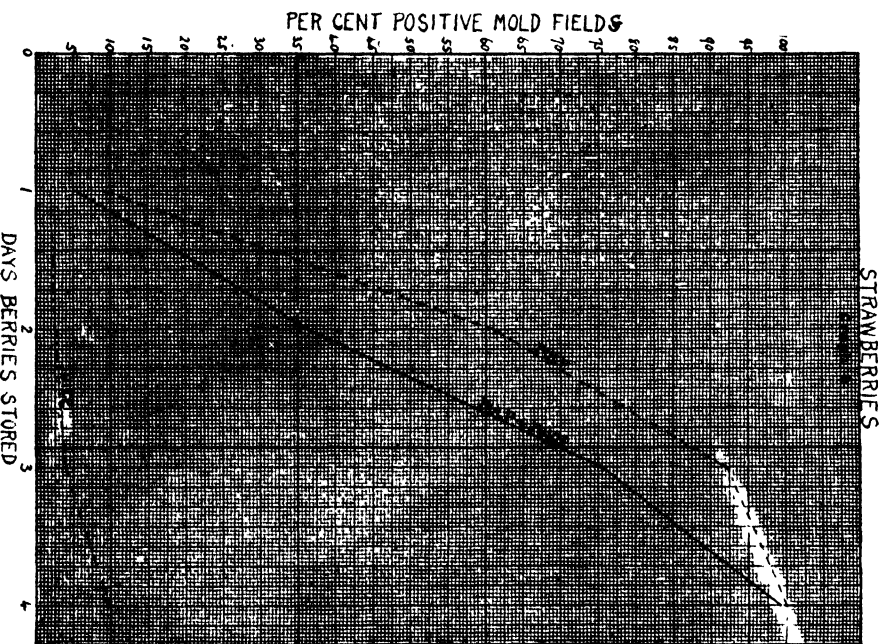
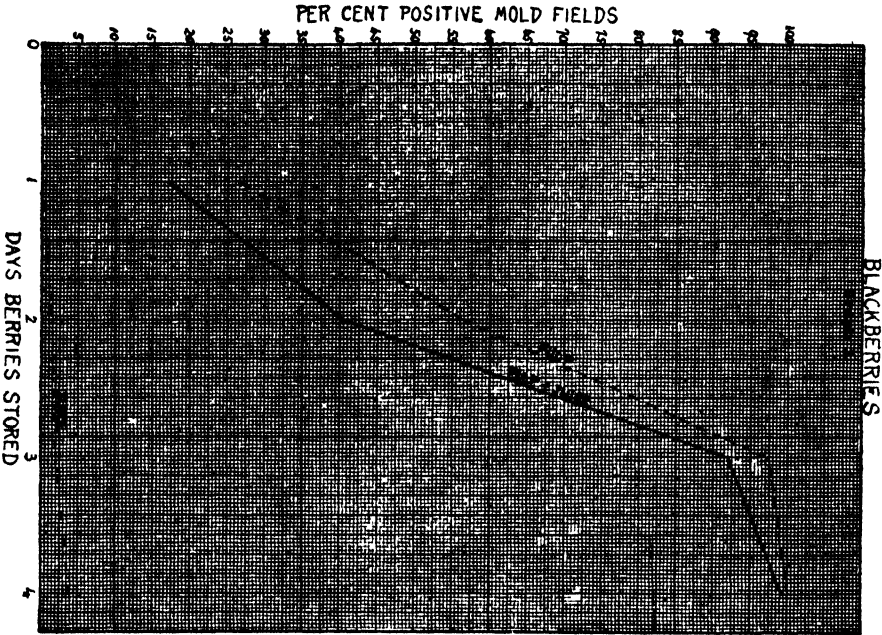


TABLE  
*Series I. Blackberries canned*

CAN NO.	TIME KEPT BEFORE CANNING	MINIMUM AND MAXIMUM TEMPERATURES OF STORAGE	BY COUNT			
			Firm	Slightly Soft	Soft	Moldy Berries
	<i>days</i>	<i>°F.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1-B	1	54-63	90.0	7.1	2.9	
	2	53-63	62.8	19.3	17.9	
	3	50-63	24.8	20.4	54.8	37.6
	4	48-63	16.3	22.8	60.9	59.8
5-B	1	53-58	66.2	22.9	10.9	
	2	50-58	58.3	23.8	17.9	
	3	48-58	26.2	23.3	50.5	51.4
	4	47-61	29.5	26.7	43.8	57.1
9-B	1	53-58	81.3	18.7	0.0	
	2	50-58	61.9	26.8	11.3	
	3	48-58	52.7	22.9	24.4	18.9
	4	47-61	37.9	24.2	37.9	40.9
Average	1		79.2	16.2	4.6	
	2		61.0	23.3	15.7	
	3		34.6	22.2	43.2	36.0
	4		27.9	24.6	47.5	52.6

*Series I. Strawberries canned*

1-S	1	54-63	77.5	17.5	5.0	
	2	53-63	44.4	25.0	30.6	
	3	50-63	31.1	15.5	53.4	24.4
	4	48-63	13.4	19.3	67.3	73.0
5-S	1	53-58	78.4	16.2	5.4	
	2	50-58	65.2	19.6	15.2	2.2
	3	48-58	35.3	20.6	44.1	26.5
	4	47-61	21.9	29.3	48.8	63.4
9-S	1	53-58	80.6	11.1	8.3	
	2	50-58	55.0	27.5	17.5	2.5
	3	48-58	25.6	25.6	48.8	11.6
	4	47-61	26.8	39.0	34.2	73.2
Average	1		78.8	14.9	6.8	
	2		54.9	24.0	21.1	1.6
	3		30.7	20.6	48.7	20.8
	4		20.7	29.2	50.1	69.9

1.

*at intervals after picking.*

JUICE		PULP		PULP AND JUICE	
Molds Positive Fields	Yeasts and Spores in 1/60 cmm.	Molds Positive Fields	Yeasts and Spores in 1/60 cmm.	Molds Positive Fields	Yeasts and Spores in 1/60 cmm
<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
0.0	12	18.0	212	14.0	136
0.0	24	44.0	380	34.0	250
0.0	81	94.0	776	90.0	311
6.0	86	100.0	783	98.0	344
0.0	21	50.0	296	32.0	154
2.0	41	82.0	469	68.0	334
2.0	81	100.0	962	100.0	452
2.0	43	100.0	584	100.0	420
0.0	14	12.0	344	6.0	188
2.0	44	44.0	672	20.0	333
4.0	67	98.0	987	86.0	375
4.0	70	100.0	1080	100.0	471
0.0	16	26.7	284	17.3	159
1.3	36	56.7	507	40.7	306
2.0	76	97.3	908	92.0	379
4.0	66	100.0	816	99.3	412

*at intervals after picking.*

4.0	36	14.0	345	8.0	154
2.0	54	48.0	414	26.0	202
6.0	70	100.0	603	96.0	248
14.0	92	100.0*	675	100.0*	285
2.0	24	8.0	378	4.0	155
4.0	36	54.0	408	30.0	206
4.0	31	80.0	420	48.0	181
10.0	41	100.0*	536	98.0	227
0.0	26	6.0	278	4.0	156
2.0	30	82.0	364	54.0	186
2.0	33	96.0	454	82.0	204
6.0	39	100.0*	648	100.0	227
2.0	29	9.3	334	5.3	155
2.7	40	61.3	395	36.7	198
4.0	45	92.0	492	75.3	211
10.0	57	100.0	620	99.3	246

\* Majority of the fields contained masses of mold.

twice through two thicknesses of muslin, and this liquid and the juice were evaporated down before the sugar was added. Both jam and jelly were boiled to 218°F. In the same way berries that had been kept two days, three days, and four days before being canned were made into jam and jelly.

*Strawberries.*—Jam only was made from the strawberries. The same process was followed as given in the previous paragraph.

The results of the counts are shown in Table 2.

#### SERIES III.

##### *Containing Definite Weights of Moldy Fruit.*

In this series berries of the finest quality were purchased and carefully sorted. However, some of the blackberries used were somewhat soft, due to the lateness of the season, making it necessary to reject half of them. Moldy berries five to six days old were added to those that were sound in definite proportions by weight. In canning, 9 parts of water were added to 17 parts of blackberries by weight; 11 parts of water were added to 12.5 parts of strawberries by weight.

The results are shown in Table 3.

#### COMPARISON OF METHODS OF SAMPLING.

A comparison of the methods of sampling yielded valuable results. It was shown that when the pulp alone was used to make the count (as is recommended by the Government), both the mold and yeast and spore counts of the good samples were consistently higher than when the combined pulp and juice were used. As the percentage of mold or rot increased, however, the results of these two empirical methods of sampling became similar, neither being accurate with very high percentages of rot. Microscopic counts made upon the juice demonstrated that regardless of the condition of the sample, the maximum mold count was 6 per cent positive fields, and the yeasts and spores 86 in 1/60 cmm. This is explained by the fact that the mold and, to a lesser extent, the yeasts and spores are in close contact with the pulp and cling tenaciously thereto. Hence, when the pulp is separated from the juice most of the mold remains with the pulp.

The tremendous difference in the results obtained by the three methods of sampling must be carefully taken into consideration in the examination of berry products. The use of the entire contents of the can is here recommended as the most suitable for general use. The following reasons are offered in support of this:

1. The entire contents of the can give the fairest sample.
2. All samples may not be drained alike, and also some may have a higher percentage of juice than others.

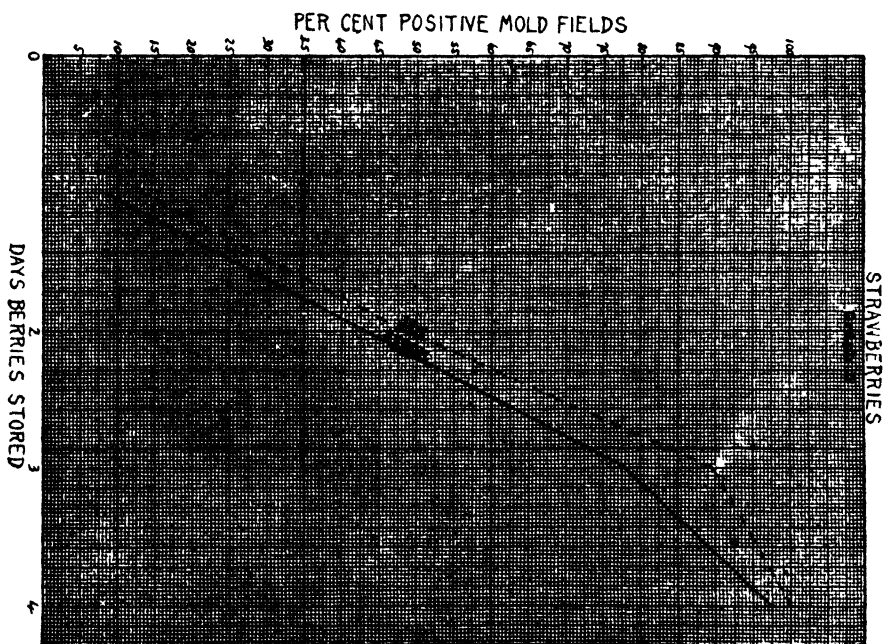
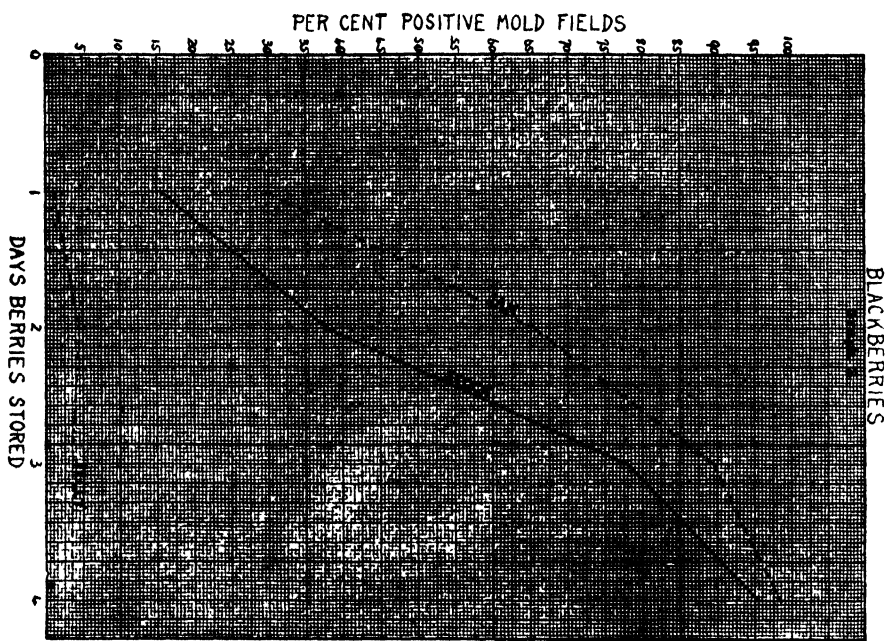


TABLE  
Series II. Blackberry jam

CAN NO.	TIME KEPT BEFORE CANNING	BY COUNT				STOCK	
						Pulp and Juice	
		Firm	Slightly Soft	Soft	Moldy Berries	Molds Positive Fields	Yeasts and Spores in 1/60 cmm.
	days	per cent	per cent	per cent	per cent	per cent	
2-B	1	86.5	10.7	2.8		16.0	342
	2	63.7	20.9	15.4		38.0	402
3-B	3	32.3	23.3	44.4	30.7	78.0	562
	4	16.5	24.2	59.3	60.8	100.0	654
6-B	1	64.2	16.5	19.3		22.0	310
	2	55.0	22.8	22.2		46.0	352
7-B	3	27.7	23.4	48.9	43.6	72.0	382
	4	35.4	26.7	37.9	42.7	88.0	410
10-B	1	78.8	20.5	0.7		8.0	240
	2	56.6	26.9	16.5		30.0	306
11-B	3	32.2	23.4	44.4	21.6	84.0	334
	4	28.0	26.1	45.9	33.8	98.0	392
Average	1	76.5	15.9	7.6		15.3	297
	2	58.5	23.5	18.0		38.0	353
	3	30.7	23.4	45.9	32.0	78.0	426
	4	26.6	25.7	47.7	45.8	95.3	485

Series II. Strawberry

2-S	1	80.0	15.0	5.0		8.0	178
	2	54.1	21.6	24.3		32.0	270
	3	20.6	19.0	60.4	23.8	82.0	286
	4	13.2	32.1	54.7	73.6	100.0*	342
6-S	1	81.8	13.6	4.6		2.0	168
	2	59.1	15.9	25.0	2.3	44.0	190
	3	48.9	22.5	28.6	26.5	58.0	202
	4	9.7	36.6	53.7	68.3	92.0	260
10-S	1	83.8	8.1	8.1		16.0	144
	2	46.7	33.3	20.0	3.3	54.0	148
	3	21.3	27.6	51.1	14.9	94.0	200
	4	22.5	32.6	44.9	63.3	100.0*	210
Average	1	81.9	12.2	5.9		8.7	163
	2	53.3	23.6	23.1	1.9	43.3	203
	3	30.3	23.0	46.7	21.7	78.0	229
	4	15.1	33.8	51.1	68.4	97.3	271

\* Majority of the fields contained masses of mold.

2.

*and jelly made from Series I.*

JAM		JELLY		REMARKS
Molds Positive Fields	Yeasts and Spores in 1/60 cmm.	Molds Positive Fields	Yeasts and Spores in 1/60 cmm.	
<i>per cent</i>		<i>per cent</i>		
18.0	322	0.0	268	Odor and taste good.
66.0	378	8.0	362	Jam, taste fair; jelly, taste good.
82.0	376	6.0	368	Jam and jelly, odor and taste good.
100.0	540	4.0	458	Jam, taste flat; jelly, taste good.
50.0	328	2.0	254	Odor and taste of both very good.
82.0	440	4.0	258	Odor and taste of both good.
92.0	334	2.0	242	Odor and taste of both good.
100.0	304	2.0	252	Jam, taste fair; jelly, taste good.
20.0	272	2.0	226	Odor and taste of both fine.
48.0	290	2.0	210	Odor and taste of both good.
94.0	298	2.0	236	Odor and taste of both good.
96.0	282	4.0	246	Jam, taste poor, but no moldy odor.
29.3	307	1.3	249	
65.3	369	4.7	277	
89.3	336	3.3	282	
98.7	375	3.3	319	

*jam made from Series I.*

26.0	140			Odor and taste fine.
44.0	176			Odor and taste good.
100.0	238			Odor and taste fair.
100.0*	258			Odor and taste moldy.
4.0	110			Odor and taste fine.
34.0	136			Odor and taste fair.
72.0	172			Odor and taste good.
100.0	232			Odor and taste fair.
10.0	104			Odor and taste good.
68.0	98			Odor fair, taste good.
96.0	138			Odor fair, taste good.
100.0*	156			Odor bad.
13.3	118			
48.7	137			
89.3	183			
100.0	215			

\* Majority of the fields contained masses of mold.



3. As is well known, the higher the concentration of the sirup used in canning berries, the greater will be the shrinkage of the fruit. It is obvious, therefore, that if the same quality of berries is canned in water and in sirup, the berries canned in sirup will give a higher mold count in the pulp, since the mold is concentrated several times during the cooking process. Furthermore, when the pulp only is examined, the juice, which is an integral part of the berry, is lost.

4. The counting of molds is greatly facilitated when both the pulp and juice are used, as in that case the microscopic fields are less dense and contain less debris than when the pulp alone is used; thus a more accurate count is made. This also holds true for the yeast and spore counts.

Graph 1 contrasts the curves of the average percentage of positive mold fields given by blackberry juice, pulp, and the mixed pulp and juice.

TABLE 3.

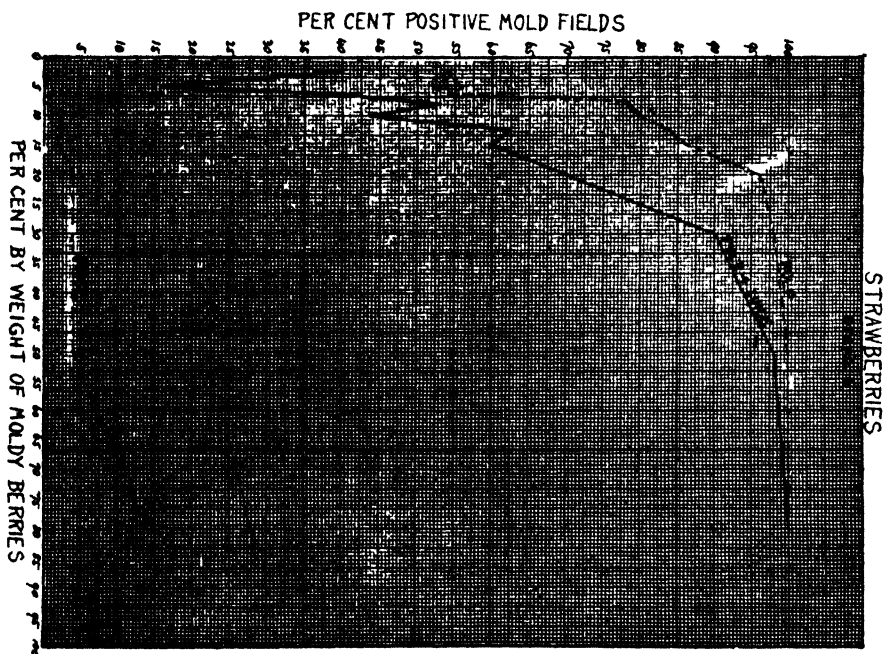
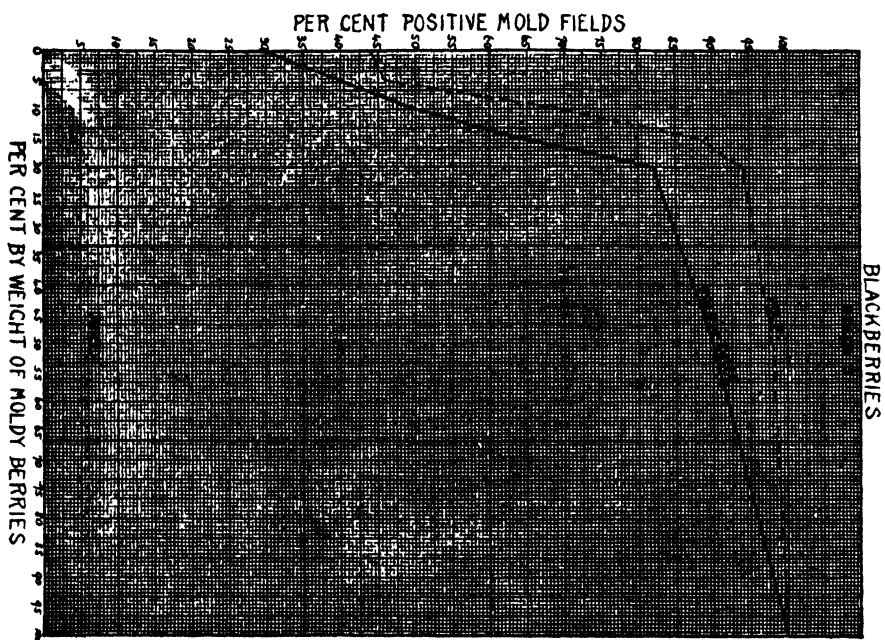
*Series III. Blackberries containing definite weights of moldy fruit.*

MOLDY BY WEIGHT	JUICE		PULP		PULP AND JUICE	
	Molds Positive Fields	Yeasts and Spores in 1/80 cmm.	Molds Positive Fields	Yeasts and Spores in 1/80 cmm.	Molds Positive Fields	Yeasts and Spores in 1/80 cmm.
<i>per cent</i>	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
0	0	18	44	166	30	127
5	0	32	46	181	40	122
10	4	52	70	287	50	246
15	4	38	88	242	64	166
20	6	50	94	401	82	283
50	6	57	98	428	90	337
100	6	107	100	537	100	461

*Series III. Strawberries containing definite weights of moldy fruit.*

2.5	2	36	54	462	40	285
5	0	43	56	362	16	168
6	0	46	54	356	38	200
7.5	0	70	78	414	53	204
10	2	47	80	584	44	282
12.5	2	90	84	563	62	317
15	2	43	86*	386	60	182
20	4	65	96	508	70	278
30	4	84	98	757	90	314
50	4	129	100*	864	98	489
80	14	148	100*	1264	100*	586

\* Majority of the fields contained masses of mold.



## DISCUSSION OF EXPERIMENTAL WORK.

*Series I. Blackberries Canned at Intervals after Picking.*

The results (see Table 1 and Graph 1) show that blackberries canned within twenty-four hours after picking were satisfactory for canning purposes. Blackberries held for two days after picking at a temperature varying from 50°–63°F. yielded inconclusive results, as only part of the berries gave high mold counts. However, blackberries held for more than two days in storage at this temperature gave uniformly high mold counts in the pulp and in the mixed pulp and juice; in all cases the positive microscopic fields were found to be 86 per cent or more.

The original condition of the berries is apparently a factor of prime importance in determining their keeping quality. After one day the average percentage of firm berries was 79.2 per cent, whereas after four days it was only 27.9 per cent. Inversely, the average percentage of soft berries increased from 4.6 per cent after one day to 47.5 per cent after four days. Therefore, a direct relation exists between the mold count and the texture of the berries.

The yeast and spore counts also increased regularly with the time of storage, averaging 284 in 1/60 cmm. after one day and 816 after four days. These counts, therefore, ran parallel to the mold counts, and they were always higher when a considerable percentage of soft fruit was used.

*Series II. Blackberry Jam and Jelly made from Series I.*

In many ways the results obtained in Series II corroborate those just discussed under Series I, *e. g.*, the mold and yeast counts steadily increased with length of storage.

The jam gave uniformly higher mold counts than the stock (see Graph 2 and Table 2), but the yeast and spore counts remained nearly constant. In the process of manufacturing jam the high temperature used and the agitation appear to break up the masses of mold and disintegrate the mold filaments. Therefore, although more positive fields were found upon microscopic examination, by actual count the number of fields containing masses of mold were fewer.

Few or no molds were found in the jelly regardless of the condition of the stock, due to the fact that only the juice was used. Some samples of moldy berries produced jams and jellies of apparently normal odor and flavor, while in other cases the odor and flavor were materially injured.

*Series III. The Relationship of Percentage by Weight of Moldy Blackberries to the Mold Count.*

These results checked those already discussed in Series I and II on the increase obtained in mold and yeast and spore counts of the pulp over those where the combined pulp and juice were used. As in the two preceding series, very little mold was found in the juice obtained from very moldy berries.

No definite conclusions should be drawn from this preliminary experiment, although it is significant that in a sample of pulp and juice made from berries having no visible mold a count of 30 positive fields was obtained. The berries were very soft, however, and were certainly mold infected. This is an important fact and was repeatedly proved in the course of this work, particularly in the case of blackberries. Graph 3 and Table 3 show the rise of the mold count of the drained pulp, and pulp and juice together with the increase of percentage of moldy berries added. Graphs plotted from supporting data in Series 1 generally checked these results.

Upon comparing these three series of blackberries, certain facts present themselves. Berries containing 5 per cent by weight of moldy fruit gave a mold count of 40 per cent positive fields in the pulp and juice. While this count is not excessive, it is not considered good policy to can any berries containing over 5 per cent of moldy fruit by weight. Therefore, it would seem that canned blackberries should not contain over 40 per cent positive mold fields in the combined pulp and juice. No definite conclusions can be drawn, however, from this one experimental pack. Additional data upon the relationship of moldy berries to the mold count are being collected and will be presented in a later paper.

*Series I. Strawberries Canned at Intervals after Picking.*

The discussion given under blackberries applies equally well to strawberries. The results (see Table 2 and Graph 2) showed that strawberries canned within one day after picking gave very low mold counts and were very satisfactory for canning purposes. On the other hand, those held longer gave high counts and were unfit for canning purposes.

Graph 4, also Table 1, shows the curves of the average percentage of positive mold fields given by strawberry juice, pulp, and the mixed pulp and juice.

*Series II. Strawberry Jam made from Series I.*

The jam gave uniformly higher mold counts than the stock (see Table 2 and Graph 5), but the yeast and spore counts of the jam were less than those of the stock.

*Series III. The Relationship of Percentage by Weight of Moldy Strawberries to the Mold Count.*

In the sample with 80 per cent by weight of moldy berries added, the strawberry juice contained a maximum of 14 per cent positive mold fields. Graph 6 shows the rise of the mold count of the drained pulp and of the combined pulp and juice with the increase of percentage of moldy berries added.

*Commercial Berry Products.*

Table 4 is self-explanatory and requires little comment. Two of the 14 commercial samples examined were of very poor quality and must have been prepared from very moldy stock. Two other samples were rated as of fair quality, while ten were given a "good" rating.

TABLE 4.  
*Commercial berry products.*

PRODUCT	MOLDS POSITIVE FIELDS	YEASTS IN 1/50 CMM.	RATING
<i>Canned Raspberries</i>			
Sample 1 . . . . .	94	634	Very poor.
Sample 2 . . . . .	6	394	Good.
Sample 3 . . . . .	8	373	Good.
Sample 4 . . . . .	0	106	Very good.
Sample 5 . . . . .	2	213	Very good.
Sample 6 . . . . .	12	277	Good.
Sample 7 . . . . .	4	123	Good.
<i>Blackberries</i>			
Sample 1 . . . . .	4	410	Good.
Sample 2 . . . . .	12	645	Good.
<i>Strawberries</i>			
Sample 1 . . . . .	8	373	Good.
<i>Jam</i>			
Sample 1. Raspberry . . . . .	88	157	Very poor.
Sample 2. Raspberry . . . . .	32	236	Fair.
Sample 3. Raspberry . . . . .	40	206	Fair.
Sample 4. Strawberry . . . . .	4	128	Very good.

## SUMMARY.

1. The microscopic count of molds, and yeasts and spores by the Howard method can be applied to berries and berry products to determine the character and quality of these products.

2. These experiments indicated that strawberries or blackberries should not be held more than a day under ordinary cannery conditions.

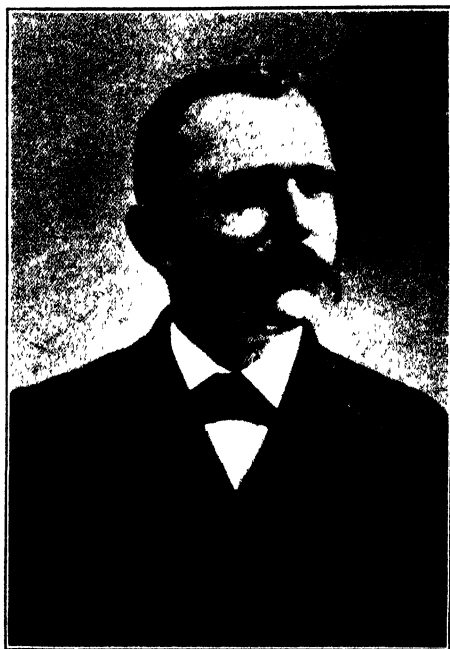
3. In every case a high mold count in fresh or canned blackberries or strawberries indicated a high percentage of soft or moldy fruit; similarly high yeast counts indicated spoiled and fermented berries, and in general high mold and high yeast counts occurred together.

4. A comparison of methods of sampling canned berries and berry products was made. The results show that the highest mold counts are obtained when the pulp alone is used as a sample. The juice was found to contain very little mold, while the count obtained on the contents of the entire can, consisting of both pulp and juice, was always somewhat lower than that of the pulp. The use of the entire contents of the can is recommended as the most suitable.

5. When jelly was made from blackberries, it was found to contain only a small amount of mold, or of yeast and spores, regardless of the condition of the raw product. On the other hand, when jam was made from blackberries or strawberries, the mold count of the finished product usually showed an increase in proportion to the mold in the raw material. The yeast count, however, showed little change except possibly a tendency towards a decrease.

6. Preliminary experiments indicate that blackberries or strawberries containing from 5 to 10 per cent of decayed or moldy berries by weight give 40 per cent or more positive mold fields in the combined pulp and juice.

7. It was found that a moderately high mold count may be obtained on soft, mushy, or over-ripe fruit which shows no readily visible evidence of mold. This is particularly true of blackberries.



WILLIAM CARTER STUBBS, 1843—1924

## WILLIAM CARTER STUBBS

Dr. William Carter Stubbs, a charter member of the Association of Official Agricultural Chemists and a pioneer in the field of agricultural research in the South, died at his home in New Orleans, La., on July 7th, 1924, after an illness with pneumonia of less than a week's duration.

Dr. Stubbs was born in Gloucester County, Virginia, on December 7th, 1843, and so was in his 81st year at the time of his death.

He was for a while a student at William and Mary College, Va., later attending Randolph-Macon College and the University of Virginia, from which latter institutions he received the degrees of B. A. and M. A. His college education was interrupted by the war, and he served with courage and fidelity as a confederate cavalryman in the army of Northern Virginia until the surrender at Appomattox in April, 1865.

In 1869 he was called to the Chair of Chemistry and Natural Science in the East Alabama College at Auburn, Ala., which three years later became the State Agricultural and Mechanical College and still later the Alabama Polytechnic Institute. Soon after the establishment of the Land Grant College, Dr. Stubbs fitted up and arranged one of the best equipped chemical laboratories in the South, and many students who received their training under him went forth to fill positions of importance and responsibility in the fields of chemistry and agriculture.

He was active in formulating and drafting legislation providing for the establishment of the State Chemical Laboratory, for fertilizer control, and of the Alabama Agricultural Experiment Station, which commenced to function nearly five years in advance of the passage of the Hatch Act. As chemist of the experiment station, he conducted investigations along a number of lines of importance to the agriculture of Alabama, including a chemical study of the sugar cane plant, and as state chemist not only directed with energy and ability the fertilizer control work of the state, but also carried out important chemical investigations as to its manurial resources, including a study of the composition of the phosphates and greensands of Alabama, in collaboration with the state geologist, Dr. Eugene A. Smith.

In the summer of 1885, Dr. Stubbs was called to Louisiana to accept the position of Director of the Sugar Experiment Station and Professor of Agriculture at the Louisiana State University, the duties of the position of State Chemist of Louisiana being also assigned him. He entered upon the work of his new position with energy and enthusiasm, and the establishment of the Sugar Experiment Station near New Orleans was soon followed by the location of the State Experiment Station at Baton Rouge and the North Louisiana Station at Calhoun.

From the first he commanded the active support and cooperation of the Louisiana Sugar Planters Association, the personnel of whose member-



ship ranked high in alertness, intelligence, and progressiveness, and within an incredibly short period of time farmers and planters engaged in other lines of agriculture likewise began to look to Dr. Stubbs with enthusiastic interest for counsel and aid in solving their various problems.

About 1890 there was established under his direction in New Orleans, at Audubon Park (the new home of the Sugar Experiment Station), the Audubon Sugar School for the purpose of training young men in the scientific, as well as the practical, phases of sugar cane culture and sugar manufacture, and from this school have gone out numbers of men that have had a conspicuous and notable part in the development and progress of the cane sugar industry not only in this country, but also in Cuba, Porto Rico, Central and South America, Hawaii, Japan, the Philippines, etc.

He was one of the founders of the "Louisiana Planter and Sugar Manufacturer" and was a member of its editorial staff until his death, while it was due to his initiative and active furtherance of the project that the Louisiana State Museum was established, and it was under his direction that a large part of this important and comprehensive collection was assembled.

As the commissioner from his state at a number of important expositions, including those at St. Louis and Jamestown, he succeeded in gathering extensive exhibits of the agricultural, mineral, and forest products of the state, and much of this material has been preserved as a permanent display illustrative of the natural resources of Louisiana.

In 1900, Dr. Stubbs was commissioned by President McKinley to visit Hawaii for the purpose of noting conditions in the cane sugar industry in those islands and locating a sugar experiment station, which commission was so well discharged as to elicit the commendation of the president.

As a result of field, laboratory, and factory tests conducted over a number of years and involving the investigation of many varieties of cane brought in from various countries, Dr. Stubbs finally recommended the planting of the variety of cane known as D 74, which has practically superseded every other variety of cane planted in Louisiana, with much resultant profit to the sugar growing industry of that state.

Many of the results of Dr. Stubbs' investigations will be found in bulletins and reports issued by the experiment stations of Alabama and Louisiana, while his work, "Sugar Cane", is regarded as an authoritative treatise on that important subject.

Dr. Stubbs was present at the preliminary meeting of official chemists at Atlanta in May, 1884; he also attended the initial meeting of the Association of Official Agricultural Chemists at Philadelphia in September, 1884, and was present at the second, third, fourth, fifth, seventh, eighth, tenth, and twelfth annual meetings of the association. He was a member of the first committee appointed to study methods for the determination of phosphoric acid, later serving as chairman of the same committee, while as chairman of the Committee on Methods of Sugar Analysis he presented the first report on that subject to the association. Thereafter, although seldom actively participating himself in the affairs of the association, he

kept in touch with the progress of its work through the members of his staff that attended the meetings and through the reports of proceedings, in which he manifested much interest.

In 1905, after twenty years of distinguished official service in Louisiana, Dr. Stubbs retired from the active work of the positions that he had so efficiently and capably filled since becoming a citizen of that state, but continued to maintain a strong and abiding interest in the progress and growth of agriculture in the state, retaining his active official connection with the Louisiana Sugar Planters Association, the American Sugar Cane League, and with the Editorial Board of the Louisiana Planter and Sugar Manufacturer.

In the quiet of his hospitable home in New Orleans, much of the time of Dr. Stubbs was spent in collaborating with his cultured wife, Mrs. Elizabeth Blair Stubbs, in historical and genealogical work involving the collection and arrangement of the records of some of the best known families of Virginia, Alabama, Louisiana, and other states of the South, the results of these labors having been published in several volumes. Indeed, it is probable that no more extensive or painstaking collections of family records than these have been made by any genealogists in the South, or even in the country at large.

The following words from the pen of Mr. Reginald Dykers, for many years secretary of the Louisiana Sugar Planters Association, give some estimate of the part played by Dr. Stubbs in the progress and development of the cane sugar industry in Louisiana:

"Although not himself a sugar planter, Dr. Stubbs was easily the most outstanding figure in connection with the sugar industry of Louisiana since Etienne de Bore, its founder. During the twenty years of his official identification with sugar production here, from 1885 to 1905, the whole status of the industry underwent a transformation and became modernized to a degree that put Louisiana, at that time, far in the lead of every other cane sugar producing country in the world in a scientific, agricultural and mechanical sense. Those two decades were a virile period in the history of our sugar industry. They were distinguished by an eager thirst for knowledge on the part of the Louisiana sugar planters and the well-spring at which they gathered and drank their fill was the Louisiana Sugar Experiment Station, of which Dr. Stubbs was the organizer and of which he was the directing head from its inception in 1885 until he retired from active service at the end of 1904.

"Magnetic, influential, brilliant, beloved, he led the Louisiana sugar planters for twenty years, not to any promised land, but to the intelligent tasks that he knew would make their own land a land of promise. He was in an industrial sense their pillar of cloud by day and their pillar of fire by night."

Spending the evening of his life in the library of his home in New Orleans among his books and historical papers, Dr. Stubbs enjoyed contact and association with scores of friends prominent in the literary, scientific, and industrial life of his city and state, and he at all times cordially welcomed

to his home many old time friends from other states and sections of the country, for friends he had almost without number.

Old students and former subordinates of Dr. Stubbs were especially received with warm greetings and open-hearted hospitality, and he followed the progress and careers of these men with an interest and solicitude that were parental in depth and sincerity, while his words of encouragement and cheer were to them an inspiration, as the writer can well testify.

Of him it can be truly said:

"His life was gentle, and the elements  
So mix'd in him that Nature might well stand up  
And say to all the world, 'This was a man'".

B. B. Ross.

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## ANNOUNCEMENT TO SUBSCRIBERS.

For some years the volume numbers of *The Journal* have not run with the calendar year, the first number of a given volume being issued in August of one year and the fourth, or last, in May of the following year. It has been decided to make the volume numbers coincident with the calendar year so that the complete proceedings of any regular annual meeting of the association may be found in one volume, the four numbers of which would issue during the calendar year immediately following that meeting.

The best way to bring this change about appears to be to put out the August and November 1925 issues as extra numbers (5 and 6) of Volume VIII and make the February 1926 issue No. 1 of Volume IX, as shown below:

Issues of. . . . .	May 1925	Aug. 1925	Nov. 1925	Feb. 1926
Old order. . . . .	Vol. VIII No. 4	Vol. IX No. 1	Vol. IX No. 2	Vol. IX No. 3
New order. . . . .	Vol. VIII No. 4	Vol. VIII No. 5	Vol. VIII No. 6	Vol. IX No. 1

Volume VIII, therefore, will contain six numbers, and binding should be deferred until the two extra numbers have been received.

R. W. BALCOM,  
R. B. DEEMER,  
W. F. HAND,  
R. E. DOOLITTLE,  
H. D. HASKINS,  
*Board of Editors.*



## FIRST DAY. MONDAY—MORNING SESSION.

### REPORT ON WATERS, BRINE, AND SALT.

By C. H. BADGER (Bureau of Chemistry, Washington. D. C.),  
*Referee.*

This year the referee studied the effect of organic matter, sodium bicarbonate, sodium chloride, sodium sulfate, and magnesium sulfate on the determination of hydrogen sulfide, paying special attention to the effect of the concentration of hydrogen ions. Since synthetic samples containing hydrogen sulfide are not stable, no collaborative work was attempted, but solutions containing relatively large quantities of hydrogen sulfide were prepared and analyzed by the referee in the Water and Beverage Laboratory of the Bureau of Chemistry.

Preliminary experiments showed that solutions that contained about 100 milligrams per liter of hydrogen sulfide could not be aerated or even poured without causing loss of hydrogen sulfide, especially when the pH values were low. In the method finally adopted, 50 cc. samples of a well mixed stock solution were carefully siphoned from a 2 gallon demijohn into 100 cc. Nessler tubes, care being taken to keep the rubber outlet of the siphon just below the surface of the sample. To each 50 cc. sample was added 5 cc. of a solution of organic matter or 5 cc. of various salt solutions. Blanks were run, 5 cc. of distilled water being used instead of the solutions. The samples and blanks were carefully mixed with a bent glass rod. To these solutions slightly less or slightly more 0.025 *N* iodine solution was added than was necessary to combine with the hydrogen sulfide, and the titration was finished, after the addition of 5 cc. of starch solution, with 0.025 *N* iodine solution or with 0.025 *N* sodium thiosulfate solution. Corrections for the blue end point were made when the titration was completed with iodine solution. The solution of organic matter was prepared by boiling dried leaves with water for a few minutes and filtering the boiled solution. The oxygen required figure, which is a measure of organic matter, was high, being 43 milligrams per 5 cc. The salt solutions were of such strength that 5 cc. contained 250 milligrams of the acid radical.

Samples 1-12 show that when the pH value of the hydrogen sulfide solution is around 5, the organic matter and salts that were added had no appreciable effect on the determination of hydrogen sulfide. It is evident that the slightly higher results in Sample 1 are of no significance, as they were not confirmed by Samples 6, 7, and 8; likewise, the slightly higher results shown by Sample 2 were not confirmed by Sample 9. Samples 13-22 show that the addition of organic matter and salts in

the quantities used has no appreciable effect even when the pH value of hydrogen sulfide solution is as low as 2.6. This pH value was obtained by adding a small quantity of hydrochloric acid to the solution. The results obtained are somewhat more variable than in solutions having higher pH values. These solutions also lost hydrogen sulfide more rapidly.

Samples 23, 24, 28, 29, 30, 36, 40, 41, 42, and 43 show that, with the exception of sodium bicarbonate, the addition of organic matter and salts has no appreciable effect even when the pH value of the hydrogen sulfide solution was increased to 8.9 by the addition of sodium hydroxide. Samples 24 and 36 show that the addition of sodium bicarbonate gives high results, but when the sample is made neutral to phenolphthalein indicator, as shown by Samples 26, 27, 38, and 39, the results obtained are satisfactory. While the stock hydrogen sulfide solution (pH 8.9) used in Samples 25 and 37 was slightly pink to phenolphthalein, no appreciable change occurred in the results when the solution was made neutral to that indicator. However, much less acid was used than when the sodium bicarbonate solution had been added.

Samples 31-35 and 44-48 show the effect of changing the pH value of the hydrogen sulfide solutions from about 8.9 to about 1.8 by the addition of one drop of dilute hydrochloric acid (1 + 1) to 50 cc. samples. The discrepancy is slight, the results being about 5 per cent lower. It should be noted that it is the addition of the acid rather than the salts that affects the results.

The pH value of the samples to which sodium bicarbonate solution was added was increased to 9.0-9.2, as shown by Samples 2, 9, 14, 19, 24, and 36, but, as noted previously, only Samples 24 and 36 gave different results. In Samples 24 and 36 the pH value of the hydrogen sulfide solution was high, and in the others part at least of the sodium bicarbonate must have been used up in neutralizing the acidity. Organic matter and the other salts used caused only a comparatively slight change in the pH value of the samples. It may be concluded that when solutions containing hydrogen sulfide have pH values from approximately 3.0-9.0, the results obtained will be satisfactory. However, if these limits are exceeded, the results will be too low or too high. Adding one drop of dilute hydrochloric acid (1 + 1) to the iodine solution did not affect its titration with sodium thiosulfate.

*Effect of certain substances on the determination of hydrogen sulfide.*

(50 cc. sample used)

LABORATORY No.*	TEMPERATURE	SOLUTIONS ADDED†	PH OF SOLUTION TITRATED	H <sub>2</sub> S PRESENT‡	H <sub>2</sub> S FOUND	H <sub>2</sub> S FOUND, AVERAGE
				mg. per liter	mg. per liter	mg. per liter
	°C.	5 cc.				
1	22	Organic matter.....	5.1	84.6	87.6; 88.2; 86.5	87.4
2	22	Sodium bicarbonate.....	9.2	85.0	88.2; 87.1;	87.6
3	22	Sodium chloride.....	5.1	85.0	85.9; 87.1; 85.9	86.3
4	22	Sodium sulfate.....	5.2	84.8	83.1; 84.8; 85.3	84.4
5	22	Magnesium sulfate.....	5.3	83.8	84.8; 85.3; 85.3	85.1
6	22	Organic matter.....	5.1	84.6	85.0; 85.3; 84.7	85.0
7	22	Organic matter.....	4.7	122.2	122.2; 124.3; 120.4	122.3
8	22	Organic matter.....	4.7	114.3	113.5; 113.5	113.5
9	22	Sodium bicarbonate.....	9.2	113.3	113.2; 113.2	113.2
10	22	Sodium chloride.....	4.7	113.5	112.8; 111.8; 112.1	112.2
11	22	Sodium sulfate.....	4.8	112.6	112.5; 111.4	111.9
12	22	Magnesium sulfate.....	4.9	111.2	111.8; 110.4	111.1
13	24	Organic matter.....	2.5	106.1	106.5; 107.1	106.8
14	24	Sodium bicarbonate.....	9.0	104.8	103.1; 104.2	103.6
15	24	Sodium chloride.....	2.5	103.7	104.8; 104.2	104.5
16	24	Sodium sulfate.....	2.6	103.6	104.2; 102.5	103.3
17	24	Magnesium sulfate.....	2.7	99.6	102.0; 104.2	103.1
18	24	Organic matter.....	2.5	97.3	98.6; 96.9	97.7
19	24	Sodium bicarbonate.....	9.0	98.0	97.9; 96.5	97.2
20	24	Sodium chloride.....	2.5	96.0	94.4; 94.8	94.6
21	24	Sodium sulfate.....	2.6	94.4	94.4; 94.1	94.2
22	24	Magnesium sulfate.....	2.7	93.3	93.4; 92.7	93.0
23	24	Organic matter.....	8.8	102.0	101.4; 102.5	101.9
24	24	Sodium bicarbonate.....	9.2	102.0	111.1; 112.8; 110.6	111.5
25	24	0.05 N hydrochloric acid§....	7.4	102.0	102.0; 102.5	102.2
26	24	Sodium bicarbonate and 0.05 N hydrochloric acid§....	7.8	102.0	101.4; 101.4; 101.4	101.4
27	24	Sodium bicarbonate and (1+1) hydrochloric acid**	8.0	102.0	100.8; 101.4	101.1
28	24	Sodium chloride.....	8.9	102.2	104.2; 102.0; 100.8	102.3
29	24	Sodium sulfate.....	8.9	102.5	103.7; 102.5	103.1
30	24	Magnesium sulfate.....	8.9	103.1	101.4; 103.7	102.5
31	24	(1+1) hydrochloric acid**	1.8	101.4	96.2; 96.8; 95.7	96.2
32	24	Organic matter and (1+1) hydrochloric acid**	1.9	101.4	96.2; 96.8	96.5
33	24	Sodium chloride and (1+1) hydrochloric acid**	2.0	101.4	96.2; 97.4	96.8
34	24	Sodium sulfate and (1+1) hydrochloric acid**	2.6	101.4	95.1; 95.7; 96.8	95.9
35	24	Magnesium sulfate and (1+1) hydrochloric acid**	2.4	101.4	95.7; 96.2	95.9
36	27	Sodium bicarbonate.....	9.2	100.5	108.0; 108.0; 108.0	108.0
37	27	0.05 N hydrochloric acid§....	7.6	100.8	100.3; 100.3	100.3
38	27	Sodium bicarbonate and 0.05 N hydrochloric acid§....	7.8	100.8	101.0; 100.0; 101.7	100.9
39	27	Sodium bicarbonate and (1+1) hydrochloric acid**	8.0	100.8	100.0; 99.6	99.8
40	27	Organic matter.....	8.9	101.3	100.0; 99.6; 101.4	100.3
41	27	Sodium chloride.....	8.9	100.8	101.4; 101.7	101.5
42	27	Sodium sulfate.....	8.9	101.2	102.1; 100.7	101.4
43	27	Magnesium sulfate.....	8.9	100.1	100.3; 101.7	101.0
44	27	(1+1) hydrochloric acid**	1.8	100.1	96.5; 95.8	96.1
45	27	Organic matter and (1+1) hydrochloric acid**	1.8	100.1	95.5; 94.8	95.1
46	27	Sodium chloride and (1+1) hydrochloric acid**	1.9	100.1	97.2; 95.5	96.3
47	27	Sodium sulfate and (1+1) hydrochloric acid**	2.6	100.1	95.8; 97.2	96.5
48	27	Magnesium sulfate and (1+1) hydrochloric acid**	2.4	100.1	95.1; 94.8	94.9

\* Titrations made with iodine solution only on Samples 1-6, 13-17, and 23-35. All other samples titrated with both iodine and thiosulfate solutions

† Five cc. solution contains 250 milligrams of the acid radical

‡ Hydrogen sulfide found when 5 cc. of distilled water was added

§ Sample made colorless to phenolphthalein

\*\* One drop of (1+1) hydrochloric acid added.



## SUMMARY.

It was found that solutions containing about 100 milligrams of hydrogen sulfide can not be agitated in the slightest degree without causing loss of hydrogen sulfide. The addition of substantial quantities of organic matter, sodium bicarbonate, sodium chloride, sodium sulfate, or magnesium sulfate to solutions of hydrogen sulfide having pH values of about 5.0 and 2.6 does not affect the results.

When the pH value of the hydrogen sulfide solution is about 8.9, the following results were found, 50 cc. samples being used: (1) Sodium bicarbonate causes high results; (2) samples containing added sodium bicarbonate made acid to phenolphthalein gave satisfactory results; (3) reducing the pH value of the solution to 1.8 by the addition of one drop of dilute hydrochloric acid (1 + 1), either with or without the addition of organic matter, sodium chloride, sodium sulfate, or magnesium sulfate, gave low results.

With the present official method not more than about 5–10 milligrams of hydrogen sulfide can be determined accurately, since the directions call for the use of only a little more than 2 cc. of 0.01 *N* iodine solution, and also owing to the loss of hydrogen sulfide due to the method of handling the sample. The referee is of the opinion that the data obtained last year and this year warrant certain modifications in the official method.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the present official method be dropped.
- (2) That the following method be adopted (first reading):

## HYDROGEN SULFIDE—OFFICIAL.

## REAGENTS.

(a) 0.05 *N* hydrochloric acid.

(b) 0.05 *N* sodium hydroxide.

(c) *Starch indicator*.—Make a thin paste with 6 grams of powdered starch and water and pour into a liter of boiling water.

(d) 0.02 *N* iodine solution.—Dissolve 10 grams of potassium iodide (free from iodic acid) in a liter flask, using as little water as possible. Add 2.54 grams of resublimed iodine and dissolve by shaking. Dilute to the mark with water. Standardize against a thiosulfate solution that has been recently standardized against a potassium dichromate solution.

(e) 0.01 *N* iodine solution.—Mix equal volumes of reagent (d) and boiled water. Standardize against a thiosulfate solution as directed under (d).

## PROCEDURE.

Transfer a measured quantity of the water to a graduated vessel by means of a siphon. Make neutral to phenolphthalein indicator with 0.05 *N* hydrochloric acid reagent (a)

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 253.

or 0.05 *N* sodium hydroxide reagent (b). Add starch indicator and, with careful stirring, titrate with iodine solution, reagent (d) or (e), until a permanent blue color appears. Correct for the quantity of iodine solution needed to give an equally blue color. From the corrected quantity of iodine solution used calculate the approximate quantity of hydrogen sulfide present. For accurate determinations siphon 100–500 cc. of the sample, according to the quantity of hydrogen sulfide present, into a graduated vessel, keeping the outlet of the siphon below the liquid. Add immediately a sufficient quantity of 0.05 *N* acid reagent (a) or 0.05 *N* alkali reagent (b), calculated from the approximate determination, to make neutral to phenolphthalein. Mix carefully with a bent glass rod and without delay add about 0.5 cc. less iodine reagent (d) or (e) than is needed to combine with the hydrogen sulfide present. Add 5 cc. of starch indicator, reagent (c), and finish the titration with iodine solution drop by drop with stirring until a blue color remains permanently. Correct for the quantity of iodine solution needed to give an equally blue color when the same quantity of starch solution is added to an approximately equal volume of boiled water. If possible, make several determinations and take an average. Standardize reagents (d) and (e) frequently.

(3) That the referee for next year make a further study of the present methods for salt<sup>1</sup>.

No report on tanning materials and leather was made by the referee.

## REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Insecticide and Fungicide Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee*.

In his report to the association at the 1923 meeting<sup>2</sup> the referee called attention to the fact that oil emulsions and miscible oils are now being used in large quantities, that their use is increasing, and that the association has adopted no methods for their analysis, and then suggested that methods of analysis for these insecticides be studied at an early date. This year's work, therefore, was devoted to the subject outlined.

It was considered that the first step in this new work should be a survey of the methods of analysis of oil emulsions and miscible oils now in use in various laboratories. Therefore, the following letter was sent to fifteen chemists in States where these insecticides are used most extensively.

The cooperative work on insecticides and fungicides for the Association of Official Agricultural Chemists for 1924 will be on methods of analysis of oil emulsions and miscible oils. In order to proceed more intelligently with this work, your advice on the following points is desired and will be greatly appreciated:

To what extent are these sprays at present used in your State and what is the probable extent of their future use?

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 309.

<sup>2</sup> *Ibid.*, 313.

What is the composition of the oil emulsions and miscible oils used at present?

What determinations do you consider necessary to be made in the examination of these products? Do you consider it necessary to determine the particular type of soap and oil used in their preparation?

Copies of the methods of analysis now in use in your laboratory and any suggestions you may have to offer will be greatly appreciated and will be of much assistance in preparing the directions for cooperative work.

Replies were received from all the chemists addressed, and an analysis of the information showed that large quantities of oil emulsions and miscible oils are used in Florida and on the Pacific coast—one California county alone using annually over 1,000,000 gallons of oil sprays—and that the use of oil sprays is increasing rapidly in other sections of the country.

The emulsifying agent in most emulsions was reported to be soap, although non-soap emulsions are also on the market.

Six chemists made suggestions as to the determinations necessary to be made. All agreed that these should include water, soap, and oil, and a majority thought that the type of oil should also be determined. In the case of miscible oils, the opinion was that the percentage of cresylic acid or tar acid should also be determined. Some suggested such determinations as loss at 100°C., ash, free alkali, and stability of emulsion on dilution.

Since the oil sprays on the market at the present time differ widely as to type of oil and emulsifier, no single procedure can be adopted for their analysis. Therefore it is necessary to resort to some classification and then to study methods applicable to a given class. Oil sprays naturally fall into the three following general groups:

First, soap emulsions;

Second, non-soap emulsions; and

Third, miscible oils.

In view of the fact that soap emulsions are the most extensively used at present, the referee decided to study them first.

#### PREPARATION OF SAMPLES.

Four oil emulsions were made as follows: Samples 1 and 3 were prepared from red engine oil according to the formula given by Ackerman<sup>1</sup>, soda fish oil soap being used in Sample 1 and potash fish oil soap in Sample 3. Samples 2 and 4 were prepared from kerosene according to the formula given by Quaintance and Siegler<sup>2</sup>, soda fish oil soap being used in Sample 2 and potash fish oil soap in Sample 4.

The quantities actually used were as follows:

<sup>1</sup> U. S. Dept. Agr. Circ. 263, p. 13.

<sup>2</sup> U. S. Dept. Agr. Farmers Bulletin 908, p. 23.

*Red engine oil emulsion (Nos. 1 and 3).*

Red engine oil.....	1 quart.
Water.....	1 pint.
Fish oil soap.....	4 ounces.

*Kerosene oil emulsion (Nos. 2 and 4).*

Kerosene.....	1 quart.
Water.....	1 pint.
Fish oil soap.....	1 ounce.

The soap was dissolved in the water; the oil was then added; and, after heating for about 10 minutes, the mixture was passed three times through a colloid mill.

The emulsions obtained were of good quality and showed no tendency toward separation of oil, though creaming out occurred after long standing.

The plans for this year being of a preliminary nature, little collaborative work seemed necessary. However, the referee did request the cooperation of the analysts in the laboratory of the California State Department of Agriculture, knowing that their experience in the analysis of oil emulsions was quite extensive. Three of the samples were sent with directions for analysis and also the suggestion that analyses be made by any methods in use in that laboratory.

The referee was also assisted by two analysts of the Insecticide and Fungicide Laboratory of the Bureau of Chemistry.

The methods sent out by the referee are as follows:

**METHOD FOR THE ANALYSIS OF MINERAL OIL—SOAP EMULSIONS.****WATER.**

Weigh approximately 25 grams of the sample into a 500 cc. flask and add 50 cc. of xylene and, if necessary to prevent foaming, a small piece of rosin. Distil into a "distilling tube receiver" of the type described by E. W. Dean and D. D. Stark<sup>1</sup>. Continue the distillation until no more water collects in the receiver.

**FATTY ANHYDRIDES.**

Weigh approximately 20 grams of the sample into a Squibb separatory funnel, add about 40 cc. each of water and ether, and thoroughly mix by gentle shaking. Break the emulsion with as small a quantity of alcohol as possible. Separate the layers and wash the lower layer twice with ether and the upper layer twice with water. Wash all the aqueous fractions with the same ether fractions that were used for the first watery layer. Combine the aqueous fractions, acidify with dilute sulfuric acid, and extract three times with ether. Wash the ether extracts from the acidified solution twice with water. Collect the washed extracts in a weighed beaker, evaporate on the steam bath, and weigh as fatty acids. To calculate fatty acids to anhydrides, multiply by the factor 0.97.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

## SODIUM OR POTASSIUM OXIDE.

Weigh approximately 10 grams of the sample into an evaporating dish, heat on the steam bath until the water is expelled, and then char the residue at a low temperature. Digest the charred mass with hot water; filter; wash thoroughly with hot water; and titrate the filtrate with 0.5 *N* sulfuric acid, using methyl orange as indicator.

## MINERAL OILS.

Determine mineral oils by difference. Check this determination by evaporating down the first set of ether extracts obtained under "Fatty Anhydrides" and weighing the residue. Care must be taken to prevent loss of the mineral oil, especially if it is a low boiling fraction.

The collaborative results are given in Table 1.

TABLE 1.  
*Collaborative results—mineral oil-soap emulsions.*

ANALYST	WATER	FATTY ANHY- DRIDES	SODIUM OXIDE	POTASSIUM OXIDE	MINERAL OIL BY EX- TRACTION	MINERAL OIL BY DIF- ERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<b>SAMPLE I</b>						
John W. Elmore, Depart- ment of Agriculture, Sacramento, Calif.	34.60	4.87	0.62	....	57.77	59.91
J. J. T. Graham	36.57	4.76	0.57	....	57.46	58.10
	36.43	4.69	0.57	..	57.76	58.31
	36.50	4.73	0.57	..	57.61	58.21
E. L. Griffin, Bureau of Chemistry, Washing- ton, D. C.	35.65	4.70	0.60	..	57.81	59.05
	36.03	4.71	0.61	..	57.86	58.65
	35.84	4.71	0.61	....	57.84	58.85
F. L. Hart, Bureau of Chemistry, Washing- ton, D. C.	35.98	5.04	0.68	....	58.37	58.30
	36.03	5.07	0.61	....	58.67	58.29
	36.01	5.06	0.65	....	58.52	58.30
General average	35.90	4.83	0.61	....	57.67	58.66
Calculated value	34.86	4.86	0.63	..	59.18	..
<b>SAMPLE II</b>						
John W. Elmore	40.40	1.25	0.16	....	31.53	58.19
J. J. T. Graham	40.56	1.36	0.16	....	55.93	57.92
	40.74	1.30	0.16	....	53.93	57.80
	40.65	1.33	0.16	....	54.93	57.86
E. L. Griffin	39.60	1.12	0.18	..	55.65	59.10
	39.48	1.07	0.17	....	54.31	59.28
	39.54	1.10	0.18	....	54.98	59.19

TABLE 1.—Concluded.

*Collaborative results—mineral oil-soap emulsions.*

ANALYST	WATER	FATTY ANHY- DRIDES	SODIUM OXIDE	POTASSIUM OXIDE	MINERAL OIL BY EX- TRACTION	MINERAL OIL BY DIF- FERENCE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
F. L. Hart	41.62	1.27	0.18	. . .	52.62	56.93
	41.42	1.27	0.19	....	54.04	57.12
	41.52	1.27	0.19	...	53.33	57.03
General average	40.54	1.23	0.17	....	51.14	58.05
	Calculated value	38.14	1.41	...	60.12	....
SAMPLE III						
John W. Elmore	36.00	3.84	....	0.73	57.57	59.43
J. J. T. Graham	37.77	3.86	..	0.70	57.95	57.67
	37.53	4.04	. . .	0.72	57.68	57.71
	37.65	3.95	..	0.71	57.82	57.69
E. L. Griffin	36.79	3.68	....	0.72	57.81	58.81
	36.95	3.67	..	0.69	58.21	58.69
	36.87	3.68	.	0.71	58.01	58.75
F. L. Hart	37.32	4.13	.	0.74	57.12	57.81
General average	37.06	3.87	. . .	0.72	57.72	58.35
	Calculated value	35.75	....	0.78	59.18	. . .
SAMPLE IV						
J. J. T. Graham	39.15	1.12	..	0.21	56.85	59.52
	39.09	1.06	..	0.21	57.54	59.64
	39.12	1.09	.	0.21	57.60	59.58
E. L. Griffin	38.75	0.83	.	0.25	57.91	60.17
	38.48	0.78	.	0.23	57.92	60.51
	38.62	0.81	....	0.24	57.92	60.34
F. L. Hart	38.97	1.41	. . .	0.27	57.00	59.35
	39.25	1.11	. . .	....	. . .	....
	39.11	1.26	...	0.27	57.00	59.35
General average	38.95	1.05	..	0.23	57.44	59.84
	Calculated value	38.41	...	0.23	60.12	. .

## COMMENTS BY ANALYSTS.

*F. L. Hart.*—The use of alcohol for breaking the emulsion increases the chance for error in that alcohol tends to remain in the ether, carrying dissolved soap along with it, thus causing slightly high results for oils and low results for fatty acids. The use of petroleum ether as a solvent would minimize this error.

The emulsion, in most cases, may be easily broken by adding 1–3 cc. of 50 per cent sodium hydroxide solution, allowing the caustic solution to run down the side of the separatory funnel, and letting the mixture stand for a short time.

*John W. Elmore.*—We believe the referee's method for fatty anhydrides gives a low result, due, probably, to the solvent action of the alcohol upon the fatty acids. It was necessary to add a considerable quantity of alcohol to break the emulsions, which even then were troublesome, particularly in working with Sample 2, the kerosene emulsion.

While the determination of oils from the ether extract is a satisfactory procedure when the oils are sufficiently heavy to be non-volatile, as in Samples 1 and 3, the results are worthless when working with emulsions containing large quantities of volatile oils, as is indicated by the percentage of oil obtained from the ether extract of Sample 2.

In response to the suggestion that comparative analyses of the samples be made by methods of the Division of Chemistry of the California State Department of Agriculture, George E. Colby and John W. Elmore submitted the following methods with the report of the results of their work using the methods sent them by the referee.

## METHODS OF ANALYSIS.

The sample should be perfectly homogeneous; the miscible oils are usually faultless in this respect, but the "mayonnaise" or non-soap emulsions sometimes show a separation of water or oil, which is reported as a defect. When small quantities are shaken up with water in a test tube the emulsion should not "break" after standing for some hours.

## DETERMINATION OF WATER.

Fifty grams is distilled slowly from a copper retort, the distillate being received in a graduated cylinder. The volume of the water layer at the bottom is read, and from this the percentage of water is calculated. It is often advisable to add xylene or kerosene to assist in bringing over the water and to prevent foaming. Sheep dips containing large quantities of oils heavier than water may be distilled with a mixture of benzene and kerosene to bring the water layer of the distillate to the bottom and give a good meniscus.

## DETERMINATION OF OIL.

*Soap Emulsions.*

Total oil is obtained by a modification of the method given in the report, "Determination of Kerosene in Kerosene Emulsions"<sup>1</sup>.

Ten grams of the thoroughly mixed sample is weighed into an 18 gram

<sup>1</sup> U. S. Dept. Agr. Bur. Chem. Bull. 105, p. 165.

Babcock cream bottle. It is then diluted with about 10 cc. of hot water, and a slight excess of sulfuric acid (1 + 1) is added. The bottle may be immersed in a hot water bath for a moment to hasten the separation and then filled with a saturated solution of sodium chloride. Place in a centrifuge and whirl at a speed of 1200 revolutions per minute for about 2 minutes and allow to cool. The volume of the oil is read, the specific gravity taken, and from these values the weight and percentage of oil in the sample are calculated. The percentage of phenols and fatty acids from the soap, which are determined separately, deducted from the percentage figure thus obtained, gives the percentage of oil in the sample.

When analyzing crude oil emulsions, it is often difficult to obtain a clear column of oil because strong sulfuric acid is likely to produce some carbonaceous matter, which collects at the bottom of the column of oil, making the reading of the volume uncertain and the specific gravity determination inaccurate. This can usually be overcome by using 5 grams of the sample instead of 10 grams and adding 5 grams of xylene. Correct for the xylene added and calculate the percentage of oil in the sample.

#### *Non-soap Emulsions.*

The Pickering type of emulsion, which contains lime water and sulfates of copper and iron, is difficult to break but it usually yields to the following treatment: 10 grams of the sample is weighed into a Babcock cream bottle as before, and an equal quantity of hot water is added. Two cc. of alcohol and 0.5 gram of sodium carbonate are then added, and the bottle is placed on a steam bath and agitated occasionally for 5-10 minutes. If the oil does not completely separate, add 1 or 2 grams more of sodium carbonate and continue the heating and agitation until the emulsion is broken. The bottle is then filled with hot water and whirled in a centrifuge. The percentage of oil is then calculated as before, but no correction is necessary for phenols.

The non-soap, "mayonnaise" type of emulsions, which depend upon gums or similar compounds as emulsifiers, are most successfully treated as follows: 10 grams of the sample is warmed and agitated in a Babcock cream bottle with 20 cc. of sodium hydroxide (1 + 1) until the emulsion is broken. Fill with sodium hydroxide (1 + 1), whirl, read the oil column, and calculate the percentage of oil in the sample.

#### DETERMINATION OF PHENOLS.

Phenols are determined by the Chapin method<sup>1</sup>.

#### DETERMINATION OF SOAP.

The soap may be determined by evaporating about 10 grams of the sample in a platinum dish, igniting to a white ash, weighing, and titrating

<sup>1</sup> U. S. Dept. Agr. Bur. Animal Ind. Bull. 107, p. 13.



with 0.1 *N* hydrochloric acid, methyl orange indicator being used. The percentage of sodium stearate or rosin soap, as the case may be, is calculated.

Another and perhaps preferable method is often followed. This method is suggested in Chemical Abstracts<sup>1</sup>.

Twenty grams of the sample is mixed in a separatory funnel with 60 cc. of petroleum ether. The mixture is then extracted with five or six 10 cc. portions of 50 per cent alcohol. The combined alcoholic extracts are washed once with petroleic ether and evaporated on a steam bath to remove alcohol. The residue containing the soap, some phenol, and other impurities is diluted with about 100 cc. of water, made alkaline with sodium hydroxide to keep the phenols in solution, cooled, and treated with an excess of saturated sodium chloride solution to precipitate the soap. After the precipitate is well coagulated it is filtered and washed with saturated sodium chloride solution. A hole is then punched in the point of the filter and the precipitate washed back into the beaker in which the precipitation was made, the filter paper being washed thoroughly with hot water. The solution is then cooled and acidified with hydrochloric acid, and the fatty acids are extracted with ether, separated, and weighed after drying, or titrated with 0.1 *N* alkali, methyl orange being used as indicator.

*Rosin in soap* may be detected by the Liebermann-Storch reaction<sup>2</sup>, and if the quantity is desired, it may be determined by the Twitchell method<sup>3</sup>.

#### UNSULFONATED RESIDUE OF OIL.

A modification of a Forest Service method<sup>4</sup> is followed, but instead of measuring the oil as therein directed 5 grams of the recovered Babcock oil is weighed into a 50 per cent standardized Babcock cream bottle. The test is then completed as directed, the specific gravity of the unsulfonated oil is taken, and the percentage by weight is obtained.

A further examination may be made by treating with caustic alkali<sup>5</sup>.

#### ASH.

Ten grams, or more if needed, is evaporated in a platinum dish, ignited to a white ash, and weighed. This ash is then tested for copper, calcium, calcium fluoride, etc.

#### STARCH, DEXTRIN, GLUE, GELATIN, GUMS, ETC.

May be detected and determined in the ether-insoluble residue after drying according to the official methods<sup>6</sup>.

<sup>1</sup> *Chem. Abstr.*, 1919, 13: 3312; *Mitt. Materialprüfungsamt*, 1918, 36: 279.

<sup>2</sup> Lewkowitsch. *Chemical Technology and Analysis of Oils, Fats and Waxes*, 5th ed., 1913-15, 1: 610. *Ibid.*, p. 625.

<sup>4</sup> U. S. Dept. Agr. Forest Service Circ. 191, p. 6.

<sup>5</sup> *Ibid.*, 206, p. 38.

<sup>6</sup> *Assoc. Official Agr. Chemists, Methods*, 1920.

Analyses by John W. Elmore of the three samples of soap-oil emulsions prepared by the referee, in which both the California and the referee's methods are used, are given in Table 2.

TABLE 2.

*Comparison of the results obtained by the referee's and the California methods on three samples of soap-oil emulsions.*

Analyst, John W. Elmore.

DETERMINATION	SAMPLE NO. 1 SODA-SOAP ENGINE OIL EMULSION		SAMPLE NO. 2 SODA-SOAP KEROSENE EMULSION		SAMPLE NO. 3 POTASH-SOAP ENGINE OIL EMULSION	
	Referee's method	California method	Referee's method	California method	Referee's method	California method
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Water .....	34.60	35.60	40.40	40.50	36.00	37.50
Fatty anhydrides. . . .	4.87	5.56*	1.25	1.25*	3.84	3.98*
Sodium oxide. . . . .	0.62	0.59	0.16	0.16	...	...
Potassium oxide . . . .	...	...	...	...	0.73	0.71
Soap (calculated from fatty acids found)....	....	6.18	..	1.40	....	4.68
Soap (calculated from al- kalinity of ash).....	....	5.88	....	1.58	....	4.85
Mineral oil by difference	59.91	....	58.19	....	59.43	..
Mineral oil from ether extract. . . . .	57.77	...	31.53	...	57.57	...
Oil .....	....	57.81	..	57.90	....	57.77
Ash, other than that of soap. . . . .	...	0.18	...	0.01	..	0.14
Unulfonated residue of separated oil . . . . .	...	65.20	..	77.20	..	63.90
Degrees Baumé. . . . .	...	29.80	...	48.10	....	29.40

\* Calculated from fatty acids.

Colby and Elmore comment as follows on the results in Table 2:

The two methods for water are practically identical, and the slightly higher result obtained by the California method is probably due to the better conduction of heat by the copper pot.

The methods for alkali oxides are practically identical. The total alkalinity calculated to soap indicated the result for fatty anhydrides by the referee's method to be low.

In the samples reported, there is a close agreement between the soap as calculated from the fatty acids and as calculated from the alkalinity of the ash. However, it would probably be more accurate, in general, to calculate the soap from the fatty acids since any free alkali contained in the soap or added as such would be included in the alkalinity of the ash.

A direct determination of the oil would seem preferable to determining it by difference, since in the latter case any errors of analysis in the other constituents are shown in the oil. Also a determination of the oil by evaporation of the ether extract is not, in general, permissible, since any low boiling oils would be lost. This is clearly shown in Sample No. 2, which is a kerosene emulsion. The centrifugal method as given in this report appears to obviate these difficulties and is in addition a rapid and economical method. For these reasons this method has been adopted in this laboratory in the analysis of oil emulsions generally.

The sulfonation test on the separated oil is useful as an indication of the nature of the oil present, the unsulfonated residue being an approximate measure of the saturated petrolic oils. This promises to become a point of great importance in the examination of oil sprays, since there is some indication that saturated and unsaturated hydrocarbons have quite different effects when applied as sprays. In at least one notable instance, an oil spray that has been unusually successful in practice in different parts of California, both north and south, shows about 95 per cent of saturated hydrocarbons in the separated oil, while others show as low as 30 per cent.

### DISCUSSION.

An examination of Table 1 shows that there is good agreement in the results for fatty anhydrides, sodium oxide, and potassium oxide. These results also agree very well with the values calculated from the referee's analysis of the soaps used in making the emulsions. In the case of water there is a maximum spread in the results of 0.77 per cent in the case of Sample 4 and 2.14 per cent in Sample 2, and all determinations with one exception are higher than the calculated values. This may be accounted for in part by the fact that in making up the samples the colloid mill was thoroughly rinsed with water before passing the mixture of soap solution and oil through it. In the determination of oil by difference all errors made in the determination of the other constituents are included in the oil. The water being high in these samples, the oil values are lower than their calculated values. The oil determination by evaporation of the ether extract was only intended as a check on the determination by difference, and, in the case of the low boiling oils, it is of no value.

### SUGGESTIONS FOR FUTURE WORK.

In the study of methods for the analysis of oil emulsions promising results were obtained, and the referee suggests that the work be continued next year. It is also suggested that a study be made of the determination of water in soaps by the xylene distillation method.

R. W. Thatcher, director of the New York Agricultural Experiment Station, has called the attention of the referee to the fact that the use of calcium carbonate as an absorbent for tobacco extract in the Kissling method<sup>1</sup> for the determination of nicotine causes a loss of nicotine by volatilization. He suggests the substitution of fullers' earth for the calcium carbonate. If this method is to be retained as an official method this point should be studied. However, it is the opinion of the referee that the silico-tungstic acid method is more preferable for nicotine determinations than the Kissling method, and that the latter should be dropped as an official method.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 65.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the study of methods of analysis of oil emulsions be continued.

(2) That the xylene distillation method for the determination of water, described in this report, be studied as a method for determining the water in soaps.

(3) That the Kissling method for the determination of nicotine in tobacco and tobacco extracts be dropped as an official method.

## REPORT ON SOILS.

By W. H. MACINTIRE (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*.

No special problem relative to soils has come to the attention of the referee during the present year. Two related subjects were assigned to the associate referees.

P. S. Burgess was appointed Associate Referee on Acidity Values of Soils. Because of a change in location, it has not been possible for him to make a report, although he has compiled a bibliography on the subject. Dr. Burgess reports that he will be glad to continue his efforts during the coming year, and he further recommends that the subject title of his refereeship be changed to "Reaction Values of Soils". In this recommendation the referee concurs.

The second line of work coming under the supervision of this referee is that of agricultural liming materials. W. M. Shaw, of the Tennessee Agricultural Station, has served as associate referee on this subject, and he has given considerable time to a study of methods for the determination of uncombined oxides and hydroxides in liming materials. His report and recommendations are to be presented. The referee also concurs in the recommendations of this associate referee.

*E. T. Wherry*.—At the time this question of the acidity of soils was taken up I thought it would be well to call attention to the great usefulness of the double-wedge apparatus for making determinations of active acidity of soils, determining the hydrogen ion by this modification of the drop ratio method. There are two principal methods, one, the use of buffer solutions and the other a modification of the drop ratio method, and then the two tubes are viewed simultaneously. The double-wedge method is simply a modification of the drop ratio method, requiring very much less trouble. The plan is to divide a rectangular cell into two parts by a diagonal partition and place on one side an acidified indicator solu-

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 253.

tion and on the other the alkaline solution of the same indicator. This can be done in glass or celluloid. You look through the cell, and at an angle or slope it gives a mixing of the two colors in different ratios. This can then be graduated on the front for each indicator, or if you want to use the same cell for different indicators, then it may be graduated in centimeters and a table of the values for the various indicators made use of. It is necessary to know very accurately the half-way point of each indicator.

I bring it up at this time because I have made numerous determinations by it on soils of all degrees of acidity, and the results have checked absolutely; they have also been checked by buffer solutions, and the agreement is as good as could be expected. There are now on the market double wedges, made up with some stabilizing solution; they will keep for several months and can then be renewed. Daily remaking of solutions is obviated, and there is not the uncertainty connected with buffer solutions. So, I thought it well to call the attention of the association to the usefulness of this method, not only with soils but a great many solutions. You can not use it for everything, but it certainly is useful for every substance for which the drop ratio has been used in the past.

No report on the acidity values of soils was given by the associate referee. The designation of this refereeship has been changed to "Associate Referee on Reaction Values of Soils".

### REPORT ON LIMING MATERIALS.

(Methods for the determination of calcium oxide and calcium hydroxide in burnt and hydrated limes.)

By W. H. SHAW (Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*.

At the last meeting of the association an Associate Referee on Liming Materials was appointed to study the relative merits of the several tentative procedures for the determination of free calcium oxide in liming materials. Carrying out the instructions, the associate referee procured a large number of lime samples from widely scattered points. From these, four hydrated and four burnt limes, both of high and low calcium oxide content, were selected. Each of the eight samples was ground to pass a U. S. Standard 60-mesh sieve, mixed thoroughly, and preserved in sealed 4 ounce bottles. A set of these samples, a copy of the tentative methods<sup>1</sup>, and the following instructions were sent out to each prospective collaborator:

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 254.

(1) All the data and comments upon the different methods should be reported to the associate referee not later than August 1.

(2) In opening the samples care should be taken to avoid introduction of chips of cork or paraffin into the material. Rubber stoppers should replace the corks as soon as the bottles have been opened.

(3) Before making any analyses the samples should be dumped upon an oil cloth or paper and carefully mixed by rolling, in order to offset any changes produced in transit. Other than this the samples are ready for analysis.

(4) The three proposed procedures are attached herewith, with the request that all the samples be analyzed simultaneously by each procedure in turn.

(5) Any deviation from the procedure outlined should be indicated in the report.

(6) A uniform style of reporting data is desirable in order to facilitate ready comparison with the results of different collaborators. Please use the form enclosed.

It was thought that the collective results and experiences of the collaborators would make a proper basis for any research that might be carried out by the associate referee. However, only three of five collaborators sent in reports, in spite of the ample time allowed—from the latter part of May to the first of August—in which to do this work. They are the following:

Leah English, N. Y. State College of Agriculture, Ithaca, N. Y.; T. D. Jarrell, Bureau of Chemistry, Washington, D. C.; and A. M. Smith, Agricultural Experiment Station, College Park, Md.

The results of the collaborative work and that done by the associate referee are shown in the tables.

TABLE 1.

*Results of determinations of available calcium oxide reported by Leah English.*

(Results reported in percentage.)

SAMPLE NO.	METHOD I*				METHOD II†				METHOD III‡			
	A	B	C	Average	A	B	C	Average	A	B	C	Average
1	38.16	38.16	38.09	38.14	39.67	39.67	39.67	39.67	39.75	39.84	39.84	39.81
2	51.06	51.06	51.06	51.06	52.33	52.33	52.47	52.38	52.39	52.39	52.28	52.35
3	37.86	37.86	37.96	37.89	39.53	39.53	39.53	39.53	39.49	39.49	39.49	39.49
4	66.15	66.20	66.20	66.18	67.83	67.83	67.83	67.83	68.23	68.23	68.34	68.27
5	50.75	50.70	50.65	50.70	52.47	52.47	52.47	52.47	52.75	52.59	52.59	52.64
6	92.60	92.60	92.58	92.59	93.23	93.28	93.28	93.26	94.26	94.26	94.26	94.26
7	52.13	52.13	52.18	52.15	53.89	53.89	53.75	53.84	53.94	53.94	53.94	53.94
8	89.12	89.12	89.12	89.12	90.44	90.38	90.38	90.40	91.21	91.21	91.21	91.21

\* Modified Stone and Scheuch.

† Modified Proctor.

‡ Modified Scaife.

*Leah English.*—Five separate analyses were made on the samples simultaneously by each procedure in turn, and those results reported which checked most closely. Considerable difficulty was experienced with Sample 2 in all three methods, Sample 7 in Method I, and Sample 6 in Method II, the first determinations made being much lower than the others.

Method II, although requiring a little longer time, is simpler in technique and gives a more distinct end point.

The averages shown in Table 1 were obtained from three concordant determinations, none of which differed from the others by more than 0.1 of one per cent by any one method. It will be seen from a comparison of the methods that II and III yield closely agreeing results on all samples except two, Nos. 6 and 8. Method I gives results about 1.6 per cent lower than those yielded by the other two methods. The possible significance that may be attached to such differences will be taken up later.

TABLE 2.

*Results of determinations of available calcium oxide reported by T. D. Jarrell.*

(Results reported in percentage.)

SAMPLE NO.	METHOD I				METHOD II				METHOD III			
	A	B	C	Average	A	B	C	Average	A	B	C	Average
1	39.8	39.8		39.8	40.1	40.4		40.3	40.0	40.2		40.1
2	53.0	53.0	52.4	52.8	54.1	53.5	53.8	53.8	55.0	54.8	54.5	54.8
3	38.5	38.5		38.5	39.5	39.5		39.5	39.0	38.8		38.9
4	66.9	67.2	67.6	67.2	68.4	68.7		68.6	68.6	68.6		68.6
5	51.4	50.9		51.2	53.0	52.7		52.9	52.8	53.0		52.9
6	93.3	93.0		93.2	93.6	93.3	93.9	93.6	94.6	94.6	94.1	94.4
7	50.4	50.0		50.2	54.1	53.8	54.1	54.0	53.0	52.8	53.0	52.9
8	90.8	90.8		90.8	90.8	91.4	91.1	91.3	92.2	91.7	91.7	91.9

*T. D. Jarrell.*—Sample No. 2 was weighed out in a small beaker for all methods, about 10 cc. of hot water was added, and the small lumps were ground with a flattened glass rod. The contents were then transferred to the flask. This was not necessary for any other sample. With this exception the methods were followed closely as outlined.

The modified Stone and Scheuch<sup>1</sup> method gives lower results than the modified Proctor<sup>1</sup> or Scaife<sup>1</sup> methods on all samples. The two latter methods give practically the same results on three samples (1, 4, and 5) and do not vary greatly on the others. The general mean of all samples by these two methods is identical, while by the modified Stone and Scheuch method it is 1.2 per cent lower.

The modified Proctor method is easier to manipulate than either of the other methods and does not require the use of so many pieces of apparatus. This is especially true when the necessary shaking is done with a mechanical shaker. This would eliminate much actual work and thus make the method very simple and short.

It is apparent from this set of results (Table 2) that closely agreeing duplicates may be obtained by any of the above procedures. The results by the Scaife and modified Proctor methods are concordant with four samples only, and with the remaining four samples the difference of the two procedures is close to 1 per cent. The modified sugar method yields results constantly lower than the other two methods, with an average difference of about 1 per cent.

<sup>1</sup> *J. Asso. Official Agr. Chemists*, 1924, 8: 254.

TABLE 3.

*Results of determinations of available calcium oxide reported by A. M. Smith.*

(Results reported in percentage)

SAMPLE NO.	METHOD I			METHOD II		METHOD III
	A	B	C	A	B*	
1	38.4	38.55		40.65	40.37	40.5
2	51.0	51.4		55.09	55.23	54.0
3	38.15	38.5		40.37	40.37	40.25
4	66.2	65.1	63.0	68.97	69.25	69.5
5	50.9	50.6		53.97	54.25	55.25
6	88.8	83.3	86.6	93.36	93.64	95.5
7	51.45	49.6	52.6	54.11	54.39	55.0
8	80.45	86.6	90.8	90.97	90.97	88.00

\* A and B are duplicate aliquots of the same weighed sample.

Since the results in Table 3 represent a single determination by Methods II and III, very little information can be drawn from them as to the capacity of these methods to yield concordant results. As to Method I, it appears to stand out in very bad light. This is particularly shown by the results on the last three samples.

## RESULTS BY THE ASSOCIATE REFEREE.

The work of the associate referee was directed towards three distinct objectives: (1) To determine the reliability of the several procedures to obtain concordance in repeated determinations; (2) to introduce certain modifications into each of the methods where such would add to the accuracy or the expediency of any of these methods; and (3) to determine the influence of impurities present in the manufactured products.

*The Scaife Method.*—This method was tried out in the regular manner and also with some modification. The modification consisted primarily in the substitution of the volumetric flask for the Pyrex Erlenmeyer flask of 600 cc. capacity. The weighed sample was introduced into the Pyrex flask, and carbon-dioxide-free distilled water, making, with the acid, a combined volume of 500 cc., was added at once; after 1 minute of agitation a volume of approximately 10 cc. of small solid glass beads was added, and from there the usual procedure was followed. These changes, it is believed, will make the prescribed shaking period more effective in breaking any lumps and also will hasten the solubility of the particles. In this regard it should be noted that the original Scaife method requires that the sample should pass a 100-mesh sieve, while as incorporated in the association's procedures this method is to be used with material that has been run through a 60-mesh sieve. The results obtained by the modified Scaife method and by the same method altered by the associate referee are given in Table 4.



TABLE 4.

*Results of determinations of available calcium oxide by two modifications of the Scaife method.*  
(Results reported in percentage.)

SAMPLE NO.	MODIFIED SCAIFE METHOD				MODIFIED SCAIFE METHOD AS ALTERED			
	A	B	C	Average	A	B	C	Average
1	39.6	39.6	40.3	39.8	40.5	40.1	40.1	40.2
2	51.2	52.4	54.1	52.9	55.3	54.2	54.2	54.6
3	38.2	38.3	39.6	39.0	40.0	39.7	39.5	39.4
4	67.1	66.2	66.8	66.7	68.6	68.1	68.2	68.3
5	53.1	54.3	49.5	52.3	53.7	53.2	53.3	53.3
6	94.2	94.5	93.1	93.9	94.5	93.6	93.5	93.9
7	50.9	49.9	47.7	49.5	53.9	54.0	53.7	53.9
8	90.4	92.1	89.9	90.8	92.1	91.2	91.1	91.5
General average				60.6				61.9

It will be seen that greater concordance, as well as greater absolute maximum, was obtained with the additional modification of this method.

*The Modified Proctor Method.*—This method was carried out as outlined in the procedures, except that the agitation was carried out in Pyrex Winchester liter flasks. The results are given in Table 5.

TABLE 5.

*Results of determinations of available calcium oxide by modified Proctor method.*  
(Results reported in percentage.)

SAMPLE NO.	A	B	AVERAGE
1	39.5	39.2	39.4
2	51.8	51.8	51.8
3	38.9	38.9	38.9
4	67.2	66.9	67.1
5	52.1	51.7	51.9
6	92.1	91.8	92.0
7	53.2	53.2	53.2
8	89.9	89.7	89.8

The results in Table 4 appear to be fairly concordant, but they are of considerably lower average than those obtained by the Scaife method.

*The Modified Stone and Scheuch Methods.*—The associate referee has been unable to carry out the method as outlined in the procedures because of temporary lack of volumetric flasks in the laboratory. It also was thought that, taking certain precautions, the sugar method might work at room temperature better than at boiling temperature.

It was considered that one of the chief sources of error and weakness of this method was the exposure of the solution to the carbon dioxide atmosphere during the filtration before titration. Accordingly, a simple device was perfected for filtration without exposure to laboratory air. The apparatus illustrated in Fig. 1 was used quite successfully in this laboratory.

The essential part of this outfit is a separatory funnel or one made by cutting off the upper end of a 25 cc. pipet just below the curvature. The edge is annealed, and the stem is somewhat drawn out and bent so as to fit into a two-holed No. 2 rubber stopper. A piece of small glass tubing is fitted into the other hole and connected with the suction pump. The funnel is closed by another two-holed stopper carrying two glass tubes; by means of rubber tubings one of these is connected with the tube reaching into the interior of the lime solution, while the other is

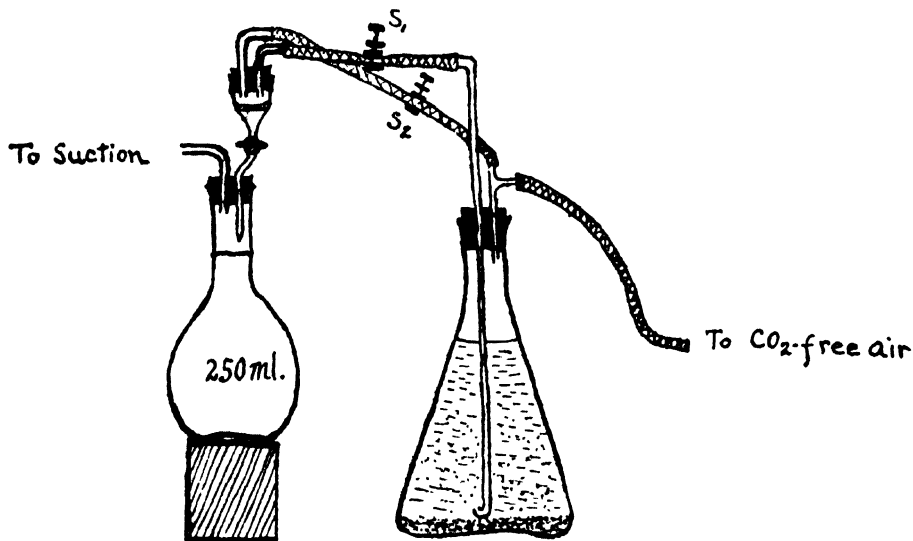


FIG. 1.—SKETCH SHOWING ARRANGEMENT FOR FILTERING LIME SOLUTION IN A CLOSED SYSTEM OF PURIFIED AIR.

connected with a T-tube, which, through one branch, receives the purified air and through the other communicates with the closed flask (holding the unfiltered lime solution).

The apparatus is connected up as shown in Fig. 1, and, with screw pinchcock  $S_2$  closed and  $S_1$  opened, the suction is started. For washing out a new filter, the cocks  $S_1$  and  $S_2$  are alternately opened and closed about six times and for rinsing the receiver an additional portion of the filtrate may be used. To keep the filter intact,  $S_1$  should be closed and  $S_2$  turned wide open whenever the receiving flask is disconnected. The filtering pad was made by placing a small cotton plug at the bottom of the funnel and covering it with about 10 cc. of a suspension of mascerated filter paper. Since the ordinary laboratory air may be contaminated occasionally with ammonia as well as with acid fumes, it is advisable to purify the air first by passing it through dilute sulfuric acid and then through granulated soda lime.

The experimental part with the sugar method was carried out to determine the effect of time on the solubility of the various lime samples in a 5 per cent sugar solution at room temperature. The results obtained from shaking the solution 5 minutes and titrating immediately, shaking 8 minutes and titrating immediately, and shaking 5 minutes and allowing to stand overnight are given in Table 6.

TABLE 6.  
*Results of determinations of available calcium oxide by modified sugar method with varying periods of shaking.*  
(Results reported in percentage.)

SAMPLE NO.	SHAKING 5 MINUTES			SHAKING 8 MINUTES			SHAKING 5 MINUTES AND ALLOWING TO STAND OVERNIGHT		
			average			average			average
1	39.8	39.6	39.7	40.0	40.0	40.0	39.2	39.2	39.2
2	54.2	53.4	53.8	54.0	54.0	54.0	53.6	53.6	53.6
3	39.6	39.6	39.6	39.8	39.8	39.8	38.8	32.0	38.9
4	68.2	67.8	68.0	68.3	68.4	68.4	67.8	67.8	67.8
5	52.0	52.0	52.0	52.5	52.3	52.4	52.0	51.8	51.9
6	93.8	93.6	93.7	93.8	94.0	93.9	93.2	93.6	93.4
7	53.6	53.6	53.6	54.3	54.0	54.2	53.4	53.4	53.4
8	90.6	90.4	90.5	92.3	91.0	91.7	90.2	90.4	90.3
			61.3			61.8			61.1

Inspection of the figures in Table 6 shows that the agreement between duplicates is as satisfactory as can be expected of any procedure. Furthermore, it shows that in a large majority of instances very little is gained from shaking 3 minutes beyond the 5 minute period. The difference in two samples is sufficient to raise the average by 0.5 per cent. Allowing the solution to stand overnight caused no increase in the results over immediate titration. On the contrary, there are indications that the additional time of standing might have caused an actual decrease in the results. This point will be taken up again.

#### SUMMARY OF COLLABORATIVE RESULTS.

The results of the collaborative work are summarized in Table 7.

Comparing the results obtained by the collaborators, it will be seen that the highest percentage of agreements was obtained with the Scaife method. Although individual analysts have been able to obtain good agreement in the general average of all determinations by the Scaife and modified Proctor methods, a closer inspection of the results on individual samples will show that those obtained by the modified Proctor method are far less concordant than those obtained by the modified Scaife method. This can be seen from the number of deviations of 0.5 per cent or more when comparing one analyst's results with the others. The total number of such deviations with the Scaife method is three, while with the Proctor method this number is fourteen. As to the modified Stone and Scheuch method, there is utter lack of agreement among

different analysts, and the results are generally lower than by the other two methods. The modifications introduced by the writer, however, seem to have overcome the difficulties in that procedure, and the results shown in the last column will stand comparison with any set of results by any procedure.

TABLE 7.

*Summary of collaborative results on available calcium oxide by the several procedures.*

SAMPLE NO.	MODIFIED SCAIFE METHOD			MODIFIED PROCTOR METHOD			MODIFIED STONE AND SCHEUCH METHOD		
	English	Jarrell	Shaw	English	Jarrell	Shaw	English	Jarrell	Shaw
1	39.8	40.1	40.2	39.7	40.3	39.4	38.1	39.8	40.0
2	52.4	54.8	54.6	52.4	53.8	51.8	51.1	52.8	54.0
3	39.5	38.9	39.4	39.5	39.5	38.9	37.9	38.5	39.8
4	68.3	68.6	68.3	67.8	68.6	67.1	66.2	67.2	68.4
5	52.6	52.9	53.3	52.5	52.9	51.9	50.7	51.2	52.4
6	94.3	94.4	93.9	93.3	93.6	92.0	92.6	93.2	93.9
7	53.9	52.9	53.9	53.8	54.0	53.2	52.2	50.2	54.2
8	91.2	91.9	91.5	90.4	91.1	89.8	89.1	90.8	91.7
Average.....	61.5	61.8	61.9	61.2	61.7	60.5	59.7	60.5	61.8
Number of instances in which the results (of the analyst) disagree with those of both the others by 0.5 per cent or more	1	2	0	3	4	7			

Thus, so far as accuracy is concerned, the chances of obtaining proper results by the methods as they stand are best with the modified Scaife method; the modified Proctor method comes next, and the modified Stone and Scheuch method last. When the filtering device and other modifications introduced by the writer are applied, the Scheuch method appears to work in a very satisfactory manner.

As regards expediency, ease of manipulation, time consumed, and other factors involved, there will naturally be some diversity of opinion as to which method should be acknowledged as the most satisfactory. Past experience with any particular method tends to make the analyst biased against other methods, equally as good or even better. The writer has already stated his conclusions concerning the accuracy of the different methods, but it may be added that in regard to expediency, it is considered that the sugar method stands out above all the other methods discussed in this report. The principal advantage is the greater speed with which an analyst can turn out any particular determination, since results may be obtained in 20 minutes after the sample has been received at the laboratory. Moreover, the labor involved is no greater than that required in the preliminary test alone by the Scaife method and much less than is required by the modified Proctor method, if a shaking machine is not available.

## ADDITIONAL WORK ON THE INFLUENCE OF ALUMINA.

As stated previously, one of the objects of this study was to determine the degree to which the results obtained by the several methods are affected by the composition of the lime sample, that is, by its content of magnesia, silica, and iron and aluminium oxides. More pressing work prevented the writer from carrying out the experimental work that would bear directly on this problem. The collaborative results, however, demonstrate that these methods are not appreciably affected by large quantities of magnesia. The general concordance secured by the various methods, when proper precautions were taken, indicates inferentially, at least, that the constituents mentioned have about the same effect on the results regardless of the procedure used. Some very glaring irregularities in connection with the modified Proctor and sugar methods, however, have been observed and noted in connection with the collaborative analyses. Some related studies with alumina in calcium hydroxide solutions that have come within the writer's observation led to the suggestion that alumina may be the cause for these irregularities. To verify this supposition, a number of determinations were made with lime samples to which varying quantities of alumina gel were added. The results of these experiments are given in Table 8.

TABLE 8.

*Results of determinations showing effect of added alumina upon the quantities of available calcium oxide, as determined by several procedures.*

SAMPLE NO.	ALUMINA ADDED $\text{Al}_2\text{O}_3$	AVAILABLE $\text{CaO}$		
		Method I (Stone and Scheuch)	Method II (Proctor)	Method III (Scaife)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
6	None	93.9	92.0	93.9
6	2.5	93.2	90.2	93.3
6	5.0	92.2	85.7	92.8
6	10.0	91.2	82.6	92.0
7	None	54.2	53.2	53.9
7	2.5	52.8	52.6	54.4
7	5.0	52.0	49.3	53.4
7	10.0	51.2	Lost	52.7

The data in Table 8 definitely demonstrate that additions of alumina cause an appreciable decrease in the percentage of available calcium oxide obtained by all procedures, but the extent of this influence varies with the different methods. With the Scaife method a 2.5 per cent addition of alumina causes a decrease of only 0.6 per cent in the available calcium oxide in Sample No. 6. With the sugar method and the modified Proctor method the corresponding decreases are 0.7 per cent and 1.8 per

cent, respectively. With the 10 per cent alumina addition the depressions in available calcium oxide by the same procedures followed in the same order are 1.7 per cent, 2.7 per cent, and 9.4 per cent, respectively. The formation of calcium aluminate,  $\text{Ca}_3\text{Al}_2\text{O}_6 \cdot 10\text{H}_2\text{O}$ , when the Proctor procedure was used in the presence of aluminium oxide, is undoubtedly the cause of such heavy depressions in the available calcium oxide. Under the imposed conditions this substance forms a very finely divided crystalline precipitate, which remains in suspension even after standing several days and is very difficultly removed by filtration. With the sugar and Scaife methods the extent of this reaction is comparatively slight, and with small quantities of alumina (1–2 per cent) it may escape detection. The other factors, in addition to the alumina content, are the concentration of the resulting calcium hydroxide solution and the time of contact. Since these factors vary more or less, it can not be expected that exactly the same depression would result from the same alumina content in different samples of commercial lime. That is, the effect of alumina on the available calcium oxide content can not be reduced to a simple mathematical formula. The Proctor method requires the longest period of contact and will always cause the heaviest depression; the results obtained by the modified sugar method and the Scaife method, as carried out by the associate referee, will be only slightly affected by the presence of 1–2 per cent of alumina.

A new question arises: Which of the two figures is more nearly correct? Is it the one that was reduced by an equivalence of the alumina present in the sample, or is it the one that was least affected by this constituent? The associate referee considers the latter value to be more nearly correct, and for the following reasons: (1) The calcium aluminate, which has been shown to be the chief cause of difference, is appreciably soluble in water; (2) in solution and in the presence of carbon dioxide this compound is readily decomposed with the formation of calcium carbonate and alumina; and (3) in the presence of active forms of silica, calcium aluminate is transformed into calcium silicate and free alumina. Thus, in so far as its utilization on acid soils is concerned, lime in the above mentioned combination should prove to be as available as calcium hydrate or calcium oxide.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the modified Proctor method be deleted as a tentative method of the association.

(2) That both the modified Scaife and the Stone and Scheuch methods, as modified by the associate referee, be continued for further study as to influences exerted by impurities in the commercial product.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 254

## REPORT ON FEEDING STUFFS.

By L. E. BOPST (State Feed Department, University of Maryland, College Park, Md.), *Referee*.

The report of the Referee on Feeding Stuffs will be incorporated in a paper written in collaboration with A. L. Flenner and O. H. Reinmuth, entitled "The Effect of Temperature and Diminished Pressure in the Determination of Moisture in Feeding Stuffs".

It is recommended<sup>1</sup> that a further study be made of the method for the direct determination of moisture by means of toluene, as described in a paper to be presented by Bidwell and Sterling<sup>2</sup>, with the idea of its adoption as an official method.

It is also recommended that the crude fiber method<sup>3</sup> be made official.

## THE EFFECT OF TEMPERATURE AND DIMINISHED PRESSURE IN THE DETERMINATION OF MOISTURE IN FEEDING STUFFS.

By L. E. BOPST, A. L. FLENNER, and O. H. REINMUTH (State Feed Department, University of Maryland, College Park, Md.).

At the meeting of this association in 1923 the referee recommended that work be done on the determination of moisture in foods and feeding stuffs. This paper describes a series of experiments in the drying of feeds.

The variety of apparatus commonly used in the determination of moisture in feeding stuffs suggested the desirability of ascertaining what influence the pressure under which samples are dried would have on the moisture values obtained.

The following feeds were employed: alfalfa meal, pure wheat bran, cottonseed meal, molasses horse feed (alfalfa, corn, oats, molasses), linseed oil meal, and a commercial chick feed. These materials were selected as representative of the general run of feeding stuffs. The molasses feed was not ground; the other feeds were passed through a 20-mesh sieve. Covered aluminum dishes, 2½ inches in diameter and 11/16 inches deep, were used in the moisture determinations.

Eight 2 gram samples of each material were weighed out, and, as a protection from the atmosphere, the dishes were placed in a sulfuric acid desiccator until all the work could be started. Complete sets were dried successively in a water-jacketed vacuum oven at 98.5°C. for 16 hours at absolute pressures ranging from 30 inches to 2 inches of mercury.

All pressures mentioned in this paper were calculated to the absolute basis: thus 30 inches of mercury pressure is equivalent to atmospheric

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 254.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 295.

<sup>3</sup> *Ibid.*, 1924, 7: 339.

pressure, and 2 inches of mercury pressure is equivalent to what is frequently described as 28 inches vacuum.

The results obtained are given in Table 1.

TABLE 1.  
*Samples dried at 98.5°C. for 16 hours at indicated pressures.*

SET	PRESSURE (INCHES)	1 ALFALFA	2 WHEAT BRAN	3 COTTON- SEED MEAL	4 MOLASSES FEED	5 LINSEED MEAL	6 CHICK FEED	GENERAL AVERAGE
A	30	8.75	10.95	7.08	11.73	9.15	10.05	9.62
B	26	8.90	11.25	7.83	12.25	10.08	10.68	10.17
C	22	9.20	11.38	7.88	12.20	10.15	10.93	10.29
D	18	8.90	11.85	8.00	12.33	10.20	11.38	10.48
E	14	9.03	11.72	7.68	12.05	10.53	11.40	10.40
F	10	9.08	11.73	7.70	12.23	10.50	11.18	10.40
G	6	9.00	11.75	7.83	11.98	10.25	11.33	10.36
H	2	9.08	11.95	8.18	12.23	10.48	11.58	10.58

It will be noted that the average moisture value obtained at a pressure of 2 inches of mercury is only 0.1 per cent greater than that obtained at a pressure of 18 inches of mercury. No individual feed shows a difference as great as 0.3 per cent. It may be safely concluded, therefore, that further diminishing of the pressure below 18 inches of mercury has little or no practical effect on the moisture values obtained.

The samples of bran, alfalfa meal, and cottonseed meal (designated as Samples 1, 2, and 3, Set D) that had been dried for 16 hours under 18 inches pressure were then further dried for 16 hours at 14 inches pressure and weighed. This procedure was repeated at pressures of 10 inches, 6 inches, and 2 inches. These weighings are given in Table 2, and a portion of Table 1 is reprinted for purposes of comparison.

TABLE 2.  
*Comparison of part of results given in Table 1 with those obtained after continued drying.*

SETS OF SAMPLES DRIED AT 98.5°C. FOR 16 HOURS AT PRESSURES INDICATED					ONE SET OF SAMPLES DRIED SUCCESSIVELY FOR 16 HOURS AT EACH PRESSURE INDICATED, TEMPERATURE 98.5°C				
Set	Pressure (inches)	1 Alfalfa	2 Wheat Bran	3 Cotton- seed Meal	Set	Pressure (inches)	Alfalfa	Wheat Bran	Cotton- seed Meal
D	18	8.90	11.85	8.00	D	18	8.90	11.85	8.00
E	14	9.03	11.72	7.68	D	14	9.03	11.98	8.10
F	10	9.08	11.73	7.70	D	10	9.18	12.00	8.13
G	6	9.00	11.75	7.83	D	6	9.25	12.00	8.13
H	2	9.08	11.95	8.18	D	2	9.45	12.03	8.23



The final average result obtained, 9.90 per cent, is only slightly higher than the average result obtained after 16 hours' drying at 18 inches pressure, 9.58 per cent, the greatest increase taking place in the alfalfa meal sample. These figures seem to indicate that after equilibrium has been attained at a pressure of 18 inches of mercury only a small amount of moisture is removed by further heating at lower pressures.

To determine whether feeding stuffs could be thoroughly dried by prolonged heating at 100°C. under atmospheric pressure, samples of alfalfa, bran, and cottonseed meal were heated to constant weight in a Freas electric oven equipped with a thermoregulator. When constant weight had been attained, the samples were further heated for 16 hours at 98.5°C. under 2 inches of pressure and weighed. The results are shown in Table 3.

The amount of moisture remaining after 125 hours of heating at atmospheric pressure is by no means large, but it is apparent that some does remain. However, the results obtained demonstrate conclusively that drying at 100°C. under atmospheric pressure does not offer a practical method of moisture determination.

Some work carried out at the Bureau of Chemistry on the determination of moisture in flours suggested a series of drying tests under atmospheric pressure at temperatures higher than 100°C.

Accordingly, duplicate samples of alfalfa meal, cottonseed meal, and wheat bran were dried at 115°, 120°, and 125°C. for periods of 1, 2, 3, and

TABLE 3.  
*Samples dried in electric oven at 100°C. atmospheric pressure.*

HOURS DRIED	ALFALFA	WHEAT BRAN	COTTONSEED
16	8.85	11.30	7.53
36	9.13	11.43	7.70
58	9.20	11.61	7.85
102	9.43	11.73	8.00
125	9.48	11.73	8.00

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*After additional drying in vacuum oven at 98.5°C. pressure,  
2 inches of mercury.*

16	9.60	11.90	8.18
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4 hours. The results were very unsatisfactory. Duplicates failed to check, and appreciable disintegration of the samples took place, as evidenced by the odors evolved. Strangely enough, most of the samples showed greater apparent moisture absorption values at the end of 3 hours' heating than at the end of 4 hours. This phenomenon has not been accounted for satisfactorily, but it must be concluded that heating at atmospheric pressure at temperatures higher than 100°C. does not offer a satisfactory method for determining moisture in general feeding stuffs, even empirically.

## CONCLUSIONS.

1. In working with feeds further diminishing of pressure below 18 inches of mercury has little or no practical effect on moisture values obtained.
2. The results given in this paper indicate that after equilibrium has been attained at a pressure of 18 inches of mercury, further heating removes only a small amount of moisture.
3. Drying feeds at 100°C. atmospheric pressure does not offer a practical method for moisture determination.
4. Subjecting samples to temperatures over 100°C. for periods of 1, 2, 3, or 4 hours affords no indication of the moisture content.

*C. H. Jones*—I have a suggestion to make. This is of particular interest to the chemists that deal with digestion trials, where the crude fiber needs to be determined in such products as feces. We found that it was practically impossible to filter the fiber after the alkali boiling and washing through linen and the crucible. It is our custom after filtering through linen and thoroughly washing with water to transfer to a 150 cc. beaker, using about 100 cc. of water. It was found practically impossible to filter this residue through the asbestos felt in a Gooch crucible. We found that by adding 50 per cent acetic acid to the residue in the beaker to make the solution approximately 0.2 per cent, and then stirring, that the material could be very quickly filtered through the Gooch crucible and as quickly washed with water and alkali, as usual. A similar concentration of sulfuric acid may be substituted. If desired, the final washing on the linen filter can be made with 0.2 per cent acetic acid and the residue transferred to the Gooch crucible directly from the linen filter. A similar procedure with cottonseed meal materially decreases the time necessary for filtration, but not to the extent noticed with feces.

*Chairman.*—Mr. Jones' comments bring to my mind an experience that I had in connection with the editing of *Methods of Analysis*, when it came to the question of the addition of asbestos in the determination of crude fiber. Apparently there is a difference of opinion among analysts as to whether or not asbestos should be used in this determination, and some very interesting data are submitted to show that its use—at least in some instances—introduces an error. I know that question was quite thoroughly covered by G. L. Bidwell and his coworkers, but apparently their conclusions are not accepted by all analysts, and I wonder if it is not worth while to have some further study on the part of the referee to determine under what conditions that error may occur.

## REPORT ON METHOD FOR THE DETERMINATION OF STARCH IN THE PRESENCE OF INTERFERING POLYSACCHARIDES.

By MAYNE R. COE (Bureau of Chemistry, Washington, D. C.), *Associate Referee on Linseed Meal.*

The variation in collaborative results last year made imperative further work on the details of the method for the determination of starch in the presence of interfering polysaccharides<sup>1</sup>. This year effort was directed toward finding a scheme for obtaining a sample free from lumps when the material is gelatinized. That feature has been overcome by the following procedure:

After the sample is washed with ether and 35 per cent alcohol and again washed with ether, it is dried sufficiently to eliminate all the alcohol present. Then as much of the dry material as possible is removed from the filter paper and placed in a glass mortar, where all lumps are pulverized. Both the filter paper and sample are then transferred to a 500 cc. volumetric flask instead of a 300 cc. flask and 20-30 cc. of distilled water is added. At this point the material should be thoroughly wetted by shaking vigorously. After standing a few minutes, 100 cc. of actively boiling water is added, and the sample is thoroughly gelatinized. Should more cold water be needed to make the material pasty, calculate the quantity of hot water to be added accordingly, so the total volume including 40 cc. of malt solution will not exceed 200 cc. Shaking occasionally obviates any local heating.

Another change in the method is suggested under the heading "Acid Hydrolysis". The statement regarding specific gravity should be amplified to read as follows: (Specific gravity 1.125 made by taking 68 cc. of concentrated acid, specific gravity 1.19 or 37.6 per cent, and diluting to 100 cc.).

### RECOMMENDATION<sup>2</sup>.

It is recommended that the changes given in this report be made in the method and that it be adopted as official.

No report on stock feed adulteration was made by the associate referee.

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<sup>1</sup> *J. Agr. Research*, 1923, 23: 995.

<sup>2</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 254.

## REPORT ON SUGARS AND SUGAR PRODUCTS.

By H. S. PAINE (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

Much progress in the conduct of the work has been made by a number of the associate referees on sugars and sugar products, and some progress, at least, has been reported by all the associate referees. The referee has kept in as close touch as possible with the associate referees in an attempt to shape the progress of the various lines of work from a unified standpoint.

The methods for sugars and sugar products are greatly in need of review and revision from the standpoint of testing and possibly adopting some of the more recent methods, eliminating certain superfluous methods, and so revising other methods as to harmonize them with recent advances in knowledge. The recommendations made in this report, together with those of the associate referees, cover many of the points involved, but there still remain a number of matters to which especial attention should be directed during the next year. The methods for the determination of reducing sugars particularly need review.

The following specific recommendations regarding revision of methods and related matters are presented for consideration:

RECOMMENDATIONS<sup>1</sup>.

(1) That Section 4, Chapter VIII<sup>2</sup>. "Drying Upon Quartz Sand—Official", be changed to provide for drying in vacuo at 70°C. in the case of products containing levulose, and that it be changed in certain details of technique. This section will then read as follows:

Digest pure quartz sand that will pass a 40-mesh but not a 60-mesh sieve with strong hydrochloric acid, wash free from acid, dry, and ignite. Preserve in a stoppered bottle. Place 25–30 grams of the prepared sand and a short stirring rod in a flat-bottomed dish approximately 60 mm. in diameter, dry thoroughly, cool in a desiccator, and weigh. Then add sufficient of the diluted sample to yield approximately 1 gram of dry matter and mix thoroughly with the sand. Heat on a steam bath for 15–20 minutes, stirring at intervals of 2–3 minutes, or until the mass becomes too stiff to manipulate readily. Dry at 70°C. under a pressure of not to exceed 100 mm. of mercury, making trial weighings at 2 hour intervals toward the end of the drying period until the change in weight does not exceed 2 mg. For materials containing no levulose or other readily decomposable substance the material may be dried at atmospheric pressure in a water oven at the temperature of boiling water, heated for 8–10 hours, cooled in a desiccator, and weighed, the heating and weighing being repeated until the loss in 1 hour does not exceed 2 mg. Report the percentage loss in weight as moisture.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 255.

<sup>2</sup> All references in these recommendations, unless otherwise designated, will be found in *Assoc. Official Agr. Chemists, Methods*, 1920, VII and VIII.

(2) That Section 9, Chapter VIII, "Refractometric Method—Official", be changed so as to provide for a standard temperature of 20°C. instead of 28°C., with the substitution of the corresponding Schönrock tables for those of Geerligs. This section will then read as follows:

Determine the refractometer reading of the solution at 20°C. and obtain the corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from table —, page —, if the instrument gives readings in terms of refractive index. If the refractometer reading is obtained at a temperature other than 20°C., correct the result according to table —, page —. If the solution is too dark to be read in the instrument, dilute with a concentrated sugar solution. Water should never be used for this purpose. Mix weighed quantities of the solution under examination and a solution of pure sugar of about the same strength, and obtain the quantity of dry substance in the former by the following formula:

$$x = \frac{(A + B) C - BD}{A}, \text{ in which}$$

$x$  = percentage of dry substance to be found;

$A$  = weight in grams of the material mixed with  $B$ ;

$B$  = weight in grams of pure sugar solution employed in the dilution;

$C$  = percentage of dry substance in the mixture  $A$  and  $B$  obtained from the refractive index; and

$D$  = percentage of dry substance in the pure sugar solution obtained from its refractive index.

(3) That Section 12, Chapter VIII, "Ash—Method III—Official" be changed in title to "Sulfated Ash" and that results be reported without correction as percentage of sulfated ash. This section will then read as follows:

Weigh 5 grams of the sample into a 50–100 cc. platinum dish, add 0.5 cc. of concentrated sulfuric acid, ignite gently until the sample is well carbonized, and then burn in a muffle at low redness to constant weight. Express the result as percentage of sulfated ash.

(4) That the recommendation of the Associate Referee on Polariscopic Methods relative to revision of Section 14, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Hydrochloric Acid—Official", be adopted.

(5) That Section 15, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase—Official—Reagent", be revised so as to conform with the most approved method for preparation of invertase. This section will then read as follows:

*Invertase solution.*—Commercial invertase preparations are available on the market. If it is desired to prepare the solution in the laboratory, the following procedure may be used. In either case the preparation may be further purified and concentrated by the ultrafiltration method described below. Commercial preparations may also be purified by dialysis and then reconcentrated by evaporating in vacuo at a temperature not to exceed 40°C.

*Preparation of crude invertase solution.*—Mix yeast with water in the proportion of 10 pounds of compressed baker's yeast with 5 liters of water. Add 2 liters of toluene and stir thoroughly at frequent intervals during the first 24 hours. Allow to stand for 7 days with occasional stirring and filter by gravity through large fluted papers. Mix the residue with 2 liters of water, filter, and combine the filtrates. Purify<sup>1</sup> by adding 15 grams of neutral lead acetate to each liter of extract and filtering on paper after all lead acetate has been dissolved. Complete the purification immediately by dialysis or by washing on the ultrafilter described below.

*Preparation of a collodion ultrafilter*<sup>2</sup>.—Dissolve 6 grams of Cooper's negative cotton (snowy) in a mixture of 50 cc. of absolute alcohol and 50 cc. of absolute ether by first adding the alcohol to the cotton, allowing the mixture to stand in a stoppered flask for 10 minutes, adding the ether, and shaking. Allow the solution to stand overnight, pour about 100 cc. into a 2000 cc. cylinder, and coat the entire inside surface of the cylinder with the collodion. Drain, and dry for 10 minutes. Fill with water, let stand 10–15 minutes, pour out the water, and remove the collodion sack. Test for leaks by filling with water. Slit open longitudinally and cut out a circular piece about 7–8 inches in diameter. Cut the bottom from a 2 liter bottle or Erlenmeyer flask and grind the edge smooth. Place it upon the still moist collodion disc, fold the edge of the disc up around the bottle, and cement it thereto with collodion that contains an increased percentage of ether. Place three or four thicknesses of wet filter paper in an 8 inch Büchner funnel. Place the bottle with the collodion membrane upon the filter paper. Pour melted vaseline, to the depth of an inch, between the bottle and inside of the funnel. Provide the bottle with a small mechanical stirring device.

*Washing and concentration of invertase solution by ultrafiltration*<sup>3</sup>.—Filter 4 liters of the partially purified solution through the ultrafilter, stirring continuously, until about 1 liter remains. Wash with distilled water introduced by means of a constant level device until the filtrate is colorless, 3 or 4 liters of wash water being required. During the entire process the invertase solution must be preserved with toluene or chinolol.

*Determining the activity of the invertase solution.*—It is generally sufficient to test the activity of the invertase solution as follows: Dilute 1 cc. of the invertase preparation to 200 cc. Transfer 10 grams of sucrose (granulated sugar) to a sugar flask graduated at 100 cc. and 110 cc., dissolve in about 75 cc. of water, add 2 drops of glacial acetic acid, and dilute to the 100 cc. mark. To the 100 cc. of sugar solution add 10 cc. of the dilute invertase solution and mix thoroughly and rapidly, noting the exact time at which the solutions are mixed. At the termination of exactly 60 minutes make a portion of the solution just distinctly alkaline to litmus paper with anhydrous sodium carbonate and determine the polarization in a 200 mm. tube at 20°C. If the invertase solution is sufficiently active, the alkaline solution will polarize approximately 31° Ventzke without correcting for the dilution to 110 cc. and the optical activity of the invertase solution.

If more exact information concerning the activity of the invertase preparation is desired, determine its velocity constant as follows: Dilute 1 cc. of the invertase solution to 200 cc. at 20°C.; place in a constant temperature bath at 20°C.; and, when the solution has attained the latter temperature pipet 20 cc. of it into a flask containing 200 cc. of a sucrose solution of 10 grams per 100 cc. concentration, that has been previously made distinctly acid to methyl red (corresponding to pH approximately 4.6) by the addition of strong acetic acid and also brought to a temperature of 20°C. in the same bath. Mix thoroughly and promptly and note the time at which the invertase solution was added. Keep the sucrose-invertase mixture in the constant temperature

<sup>1</sup> *Ind. Eng. Chem.*, 1924, 16: 562.

<sup>2</sup> *Ibid.*, 170.

<sup>3</sup> *Ibid.*, 169.

bath; remove portions at the end of 15, 30, and 45 minutes; render each portion just distinctly alkaline to litmus paper with anhydrous sodium carbonate immediately after removing; and determine the polarization at 20°C. Correct all polarizations for the polarization of the invertase solution. Calculate the velocity constant,  $k$ , for each of the polarizations (at the times  $t$ ) subsequent to the initial polarization by the following formula:

$$k = \frac{\log_{10} 1.32 R_0 - \log_{10} (R_t + 0.32 R_0)}{t}, \text{ in which}$$

$k$  = the unimolecular reaction velocity constant;

$t$  = number of minutes elapsing from the time the invertase and sucrose solutions were mixed until inversion was stopped by addition of sodium carbonate;

$R_0$  = initial polarization (calculated by multiplying the polarization of the sucrose solution by 10/11 and correcting for the polarization of the invertase solution); and

$R_t$  = polarization at time  $t$ .

An invertase solution of sufficient activity should yield an average value for  $k$  (for the various time periods) of at least 0.1 after multiplying the  $k$  value directly obtained by 200, in order to correct for the initial dilution of the invertase solution. The dilution of the invertase solution above mentioned is made solely for the purpose of determining its activity; the original, undiluted invertase solution is used as the inverting reagent in the determination of sucrose. The activity of the invertase preparation required for rapid inversion is the same as that needed for overnight inversion, but the proportion of invertase preparation used in the former case is twice that used in the latter instance.

An invertase preparation of the activity specified may be obtained from the Wallerstein Laboratories, 171 Madison Avenue, New York City.

(6) That the recommendation of the Associate Referee on Polariscopic Methods regarding revision of Section 16, Chapter VII, "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase—Official—Determination", be adopted.

(7) That the following paragraph providing for rapid inversion by invertase be added to "Determination of Sucrose in the Absence of Raffinose—By Polarization Before and After Inversion with Invertase—Official—Determination".

If more rapid inversion is desired, proceed as follows: Prepare the sample as directed above and to 50 cc. of the lead-free filtrate in a 100 cc. volumetric flask add glacial acetic acid in sufficient quantity to render the solution distinctly acid to methyl red. The quantity of acetic acid required should be determined before pipetting the 50 cc. portion as above described. Then add 10 cc. of invertase solution, mix thoroughly, place the flask in a water bath at 55°-60° C., and allow to stand at that temperature for 15 minutes with occasional shaking. Cool, add sodium carbonate solution until distinctly alkaline to litmus paper, dilute to 100 cc. at 20°C., mix well, and determine the polarization at 20°C. in a 200 mm. tube. Allow the solution to remain in the tube for 10 minutes and again determine the polarization. If there is no change from the previous reading, the mutarotation is complete. Carefully note the reading and the temperature of the solution. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the formula given. (If the solution has been rendered so alkaline as to cause destruction of sugar, the polarization, if negative, will in general decrease, since the decomposi-

tion of fructose ordinarily is more rapid than that of the other sugars present. If the solution has not been made sufficiently alkaline to complete mutarotation quickly, the polarization, if negative, will in general increase. As the analyst gains experience he may omit the polarization after 10 minutes if he has satisfied himself that he is adding sodium carbonate in sufficient amount to complete mutarotation at once without causing any destruction of sugar during the period intervening before polarization.)

(8) That the following formulas be substituted for the corresponding formulas now included in Section 17, Chapter VII, "Determination of Sucrose and Raffinose—Official", these changes being based upon a revision of the Clerget divisor and raffinose inversion constant as published by Browne<sup>1</sup>.

$$S = \frac{0.514 P - I}{0.844}, \text{ and}$$

$$R = \frac{0.33 P + I}{1.563}.$$

(9) That in Section 20, Chapter VIII, "Method II (Double Dilution Method)—Official" the portions reading "The true direct polarization of the sample is the product of the two direct readings divided by their difference" and "The true invert polarization is the product of the two invert readings divided by their difference", be respectively changed to read as follows:

The true direct polarization of the sample is equal to four times the direct polarization of the diluted solution less the direct polarization of the undiluted solution and the true, invert polarization is equal to four times the invert polarization of the diluted solution less the invert polarization of the undiluted solution.

(10) That in Sections 21 and 22, Chapter VIII, "Commercial Glucose", Methods I and II, results be reported in terms of glucose solids, the factor 211 being used instead of 175 and 196 instead of 163. The factor 196 is a rounded figure based upon the average value 195.8 found by Bryan<sup>2</sup> and 196.2 recently found by Lathrop<sup>3</sup>. This change will eliminate variations due to differences in water content of the glucose.

(11) That the various recommendations and suggestions made by the associate referees that have not been specifically mentioned in this report be adopted.

(12) That the designation of the subdivision "Maltose Products" under "Sugars and Sugar Products" be changed to "Starch Conversion Products", thereby including materials such as commercial glucose, dextrose, dextrans, etc.

(13) That the work on sugars and sugar products during the next year be conducted along the lines indicated in the reports of the various associate referees.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1921, 13: 793.

<sup>2</sup> *J. Franklin Inst.*, 1911, 172: 337.

<sup>3</sup> Unpublished.



## REPORT ON HONEY—DETECTION OF ARTIFICIAL INVERT SUGAR IN HONEY.

By WILLIAM SEAMAN (U. S. Food and Drug Inspection Station, New York, N. Y.), *Associate Referee*.

In the report of Sidney F. Sherwood, associate referee on honey, presented at the 1923 meeting of the association<sup>1</sup>, there were given the results of collaborative work, both for 1922 and for 1923, on the application to heated honeys of the resorcin and aniline chloride tests for artificial invert sugar. On the basis of this report and of that presented at the 1921 meeting of the association<sup>2</sup>, it was decided that the heating of honeys "at temperatures that would prevail in the ordinary commercial handling of this product" does not result in the production of a positive resorcin or aniline chloride test with normal honey. It appeared, however, from an examination of the 1922 samples, which had been stored until shortly before the 1923 meeting, that this conclusion—that a heated unadulterated honey would not give a positive resorcin or aniline chloride test—might not apply to heated honeys that had been stored for any length of time before their examination.

It was recommended, in view of this new evidence, that the associate referee for 1924 continue the study of heated honeys that have been stored after heating. This has been done, and there is given in this report the results of collaborative work done on heated and stored honeys.

The samples that were sent to collaborators consisted of the identical ones, portions of which had been sent out to the collaborators in 1923. They were obtained from Sherwood and had been standing at room temperature since September 12, 1923, when they were heated. A description of these samples is given below. (The acidity values, expressed as percentage of free acid as formic, are those taken from Sherwood's description of the samples.)

No. 1. Buckwheat—Acidity 0.11.

No. 2. Clover—Acidity 0.09.

No. 3. Mixture of Buckwheat and Clover. Contained added tartaric acid in the ratio of 2 grams to 500 grams of honey. Acidity 0.30.

No. 4 Honeydew—Acidity 0.13.

The samples were treated as follows:

Series A—Heated for 1 hour at 160°F. (71.1°C.).

Series B—Heated for  $\frac{1}{2}$  hour at 180°F. (82.2°C.).

Series C—Heated for 20 minutes at 208°–209°F. (97.8°–98.3°C.).

In addition to the above samples there were prepared and sent to the collaborators the following samples:

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 345.

<sup>2</sup> *Ibid.*, 1922, 5: 429.

Sample A—An invert sugar sirup prepared according to the method of Herzfeld<sup>1</sup>.

Sample X—Clover honey (acidity = 0.07) plus 20 per cent of invert sugar sirup (Sample A).

These samples were sent out in order to get additional information concerning the reliability of the tests as shown by their application to honeys known to be adulterated.

The directions given for making the tests were those of this association<sup>2</sup>, but they included more detailed information of the color reaction for a positive test. The collaborators were S. F. Sherwood, Bureau of Plant Industry, Washington, D. C., and Louis Katz, B. B. Wright, and Ray Powers, of the U. S. Food and Drug Inspection Laboratory, New York, N. Y. Two other collaborators did not turn in their results in time for inclusion in this report.

The results obtained by the collaborators are embodied in Tables 1 and 2. In most instances there has been reported not only whether the test was considered negative, suspicious, or positive, but also the color obtained. It must be remembered that a positive test is defined as a cherry red color appearing immediately (within ten seconds), and that yellow to salmon-pink shades should be ignored.

Owing, partly, to the failure of two of the collaborators to report their results, and partly to a shortage in the quantity of sample that was left over from 1923, which reduced in some instances the number of samples sent to collaborators, it is undesirable to draw final conclusions concerning the effect of storage on heated honeys. The work should be continued. Tentatively, however, the associate referee considers that there is justification for the conclusion that both with Fiehe's test (Bryan's modification) and with Feder's test there is a decided tendency for a storage period of 10–12 months after heat treatment, the period during which the samples were stored, to result in positive tests with unadulterated honeys. Whereas, in 1921, 1922, and 1923, the collaborative data on heated honeys showed practically a unanimous agreement as to the absence of positive tests, this year the heated and stored honeys show an appreciable number of positive and suspicious tests. As might be expected, the honeys of greater acidity and those subjected to a higher heat treatment tend to give a larger number of positive or suspicious tests in the hands of the collaborators than those of lower acidity or those subjected to a lower heat treatment.

In regard to the question whether a negative result with the aniline chloride and the resorcin tests is a certain indication of the absence of added invert sugar sirup, the collaborative results of 1921, 1922, 1923, and this year speak for themselves. Combining the results of the four years, it is found that with the resorcin test on blanks containing 20 per

<sup>1</sup> U. S. Dept. Agr. Bur. Chem. Bull. 110, p. 64.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 112.

cent added invert sugar sirup, out of seventeen results reported as positive, negative, or suspicious, ten were positive and seven negative; with the aniline chloride test, out of thirteen reported as positive, negative, or suspicious, seven were positive, three suspicious, and three negative. These results, it is believed, necessitate the conclusion that a negative result is not conclusive evidence of the absence of added invert sugar sirup.

For several reasons both Bryan's modification of Fiehe's test and Feder's test are not quite satisfactory. Some of the reasons are the following:

- (1) Feder's test can not be applied to dark honeys.
- (2) Both tests are inconclusive in the case of a negative result.
- (3) Both tests probably are not reliable when applied to honeys that have been heated and stored.
- (4) The tests share the disadvantage of most color reactions in requiring a certain amount of experience in their use in order to obtain correct evaluation of results.

In view of these reasons, it would be desirable to find, if possible, a test that does not have these disadvantages and that could be used either alone or in conjunction with the resorcin and the aniline chloride tests to determine invert sugar adulteration of honey.

Such a test may possibly be found in a procedure described by Auerbach and Bodländer<sup>1</sup>. They analyzed a number of natural and artificial honeys for glucose and fructose and determined the ratio of fructose to glucose. They found that in the case of natural honeys the ratio was 1.06 to 1.19; that this ratio is altered very little by heating; and that it increases upon long storage. In the case of artificial honey, the ratio is as a rule below 0.90, but in the case of such honeys as have been adulterated with glucose the ratio is much lower.

It is the opinion of the associate referee that a study should be begun to determine to what extent these conclusions are valid, and whether the procedure described can be put to practical use in the detection of adulteration of honey.

#### CONCLUSIONS.

1. The resorcin test (Bryan's modification of Fiehe's test) and the aniline chloride test (Feder's) when positive are conclusive of the presence of commercial invert sugar in honey, unless it can be shown that the honey has been stored for some length of time after having been heated to temperatures of upwards of 160°F. (71.1°C.).

2. The resorcin test and the aniline chloride test when negative are not conclusive of the absence of commercial invert sugar sirup in honey.

<sup>1</sup> *Z. Nahr. Genussm.*, 1924, 47: 233.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the following parts of the "conclusions" of the report for 1923 be made a part of the *A. O. A. C. Methods of Analysis*:

The resorcin test may be applied to all types of honey, but the aniline chloride test is of no value in the case of dark colored honey.

The statement that a positive test consists of a bright red color is insufficient for the guidance of the analyst. It should be stated that a positive test consists of a cherry red color appearing at once and that yellow to salmon shades of color have no meaning.

(2) That the "conclusions" of this report be made part of the *A. O. A. C. Methods of Analysis*.

(3) That investigation be continued of the resorcin and the aniline chloride tests on honeys that have been stored for various periods of time after having been heated to temperatures that would prevail in the ordinary commercial handling of the product.

(4) That a study be begun concerning the possibility of using the procedure of Auerbach and Bodländer in the detection of adulteration of honey with invert sugar sirup.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8, 260.

TABLE 1.  
*Resorcin test.*  
 (Bryan's modification of Fiehe's test.)  
 BLANKS.

SAM- PLE	TIME	POWERS	WRIGHT	KATZ	SEAMAN	SHERWOOD
X	Immediate	Positive	Negative (slight darken'g)	Positive	Positive (cherry red)	Positive
	1 minute	Positive (slight red)	Suspicious (deep pink)	Positive (stronger)	Positive (cherry red)	Positive
	5 minutes	Positive (red)	Positive (deep red)	Positive (dark cherry red)	Positive (cherry red)	Positive
A	Immediate	Positive	Negative (light pink)	Positive	Positive (cherry red)	Positive
	1 minute	Positive (red)	Positive (deep red)	Positive (stronger)	Positive (cherry red)	Positive
	5 minutes	Positive (dark red)	Positive (dark red)	Positive (dark cherry red)	Positive (cherry red)	Positive
SERIES A—1 HOUR AT 160°F.						
1	Immediate	Negative	Negative (slight darken'g)	Negative	Negative (colorless)	Negative
	1 minute	Positive (light red)	Negative (very light pink)	Suspicious (slight cherry tint)	Negative (pink)	Positive
	5 minutes	Positive (red)	Negative (light pink)	Positive (light cherry)	Positive (cherry red)	Positive
2	Immediate	Positive	Negative (light pink)	Positive (light cherry)	Negative (colorless)	Negative
	1 minute	Positive (pink)	Negative (light pink)	Positive (light cherry)	Negative (slight pink)	Suspicious
	5 minutes	Positive (dark pink to red)	Negative (light pink)	Positive (light cherry)	Suspicious (deep pink)	Positive
3	Immediate	Suspicious	Suspicious (light pink)	Negative (slight yellowish)	Negative (pink)	Positive
	1 minute	Positive (light red)	Suspicious (pink)	Positive	Suspicious (pink)	Positive
	5 minutes	Positive (cherry red)	Suspicious (light red)	Positive (strong cherry)	Positive (cherry red)	Positive
4	Immediate	Positive (cherry red)	Suspicious (light red)	Positive (strong cherry)	Negative (colorless)	Negative
	1 minute	Positive (cherry red)	Suspicious (light red)	Positive (strong cherry)	Suspicious (pink)	Suspicious
	5 minutes	Positive (cherry red)	Suspicious (light red)	Positive (strong cherry)	Positive (cherry red)	Positive

TABLE 1.—Continued.

*Restorcin test.*(Bryan's modification of Fiehe's test.)  
SERIES B— $\frac{1}{2}$  HOUR AT 180°F.

SAM- PLE	TIME	POWERS	WRIGHT	KATZ	SEAMAN	SEERWOOD
1	Immediate	Negative	Negative (slight darken'g)	Negative (slight yellow)	Negative (colorless)	Suspicious
	1 minute	Positive (light pink)	Negative (very light pink)	Suspicious (light pink)	Negative (pink)	Positive
	5 minutes	Positive (red)	Negative (light pink)	Positive (light cherry)	Suspicious (pink)	Positive
2	Immediate	Positive	Negative (slight darken'g)	Negative (colorless)	Negative (colorless)	Suspicious
	1 minute	Positive (pink)	Negative (very light pink)	Suspicious (light pink)	Negative (pink)	Positive
	5 minutes	Positive (cherry red)	Negative or suspicious (pink)	Positive (light cherry)	Suspicious (pink)	Positive
3	Immediate	Suspicious	Suspicious (slight brownish pink)	Slightly positive	Negative (pink)	Positive
	1 minute	Positive (pink)	Suspicious (pink)	Positive	Suspicious (pink)	Positive
	5 minutes	Positive (red)	Positive (red)	Positive (dark cherry red)	Positive (cherry red)	Positive
4	Immediate	Positive	Negative (slight darken'g)	Negative	Negative (colorless)	Suspicious
	1 minute	Positive (pink)	Negative (very light pink)	Slightly positive	Negative (pink)	Positive
	5 minutes	Positive (cherry red)	Negative (light pink)	Positive	Positive (cherry red)	Positive

## SERIES C—20 MINUTES AT 208°-200°F.

1	Immediate	Negative	Negative (slight darken'g)	Negative	Negative (colorless)	Positive
	1 minute	Positive (light pink)	Negative (light pink)	Positive	Negative (pink)	Positive
	5 minutes	Positive (red)	Negative (very deep pink)	Positive (dark cherry red)	Positive (cherry red)	Positive
2	Immediate	Suspicious	Negative (slight darken'g)	Slightly positive	Negative (colorless)	Positive
	1 minute	Positive (light pink)	Negative (very light pink)	Positive	Negative (pink)	Positive
	5 minutes	Positive (cherry red)	Negative (light pink)	Positive (cherry red)	Positive (cherry red)	Positive
3	Immediate	Positive	Negative (very light pink)	Positive	Negative (pink)	Positive
	1 minute	Positive (red)	Suspicious (light red)	Strongly positive	Suspicious (pink)	Positive
	5 minutes	Positive (cherry red)	Positive (red)	Positive (dark cherry red)	Positive (cherry red)	Positive
4	Immediate	Negative	Negative (slight darken'g)	Slightly positive	Negative (colorless)	Suspicious
	1 minute	Positive (pink)	Negative (pink)	Positive	Suspicious (pink)	Positive
	5 minutes	Positive (cherry red)	Suspicious (light red)	Positive (dark cherry red)	Positive (cherry red)	Positive

TABLE 2.  
*Aniline chloride test.*  
(Feder's.)  
BLANKS.

SAMPLE	TIME	POWERS	WRIGHT	KATZ	SEAMAN	SEERWOOD
X	Immediate					
	1 minute	Positive (cherry red)	Negative (slight darken'g)	Positive (very strong pink)	Positive (cherry red)	Positive
	5 minutes	Positive (cherry red)	Suspicious (bright pink)	Positive (very strong pink)	Positive (cherry red)	Positive
A	Immediate	Positive (cherry red)	Suspicious (bright pink)	Positive (weaker)	Positive (light amber)	Positive
	1 minute	Positive (cherry red)	Suspicious (pink tint)	Positive (cherry red)	Positive (cherry red)	Positive
	5 minutes	Positive (cherry red)	Positive (red)	Positive (cherry red)	Positive (cherry red)	Positive
	Immediate					
	1 minute	Positive (cherry red)	Positive (red)	Positive (cherry red)	Positive (cherry red)	Positive
	5 minutes	Positive (cherry red)	Positive (red)	Positive (cherry red)	Positive (dark amber)	Positive

SERIES A—1 HOUR AT 160°F.						
1	Immediate					
	1 minute	Too dark to test	Negative	Negative (pink tint)	Too dark to test	Too dark to test
	5 minutes	Too dark to test	Negative (pinkish cast)	Negative (much weaker)	Too dark to test	Too dark to test
2	Immediate					
	1 minute	Suspicious	Negative (brown)	Negative	Negative (slightly pink)	Too dark to test
	1 minute	Positive (light pink)	Negative (brown)	Negative	Negative (slightly pink)	Too dark to test
	5 minutes	Positive (light pink)	Negative (brown)	Negative	Negative (orange yellow)	Too dark to test
3	Immediate					
	1 minute	Positive	Negative	Suspicious (strong pink)	Too dark to test	Too dark to test
	1 minute	Positive (cherry red)	Positive (red)	Negative (much weaker)	Too dark to test	Too dark to test
	5 minutes	Suspicious (brown)	Suspicious (brown)	Negative	Too dark to test	Too dark to test
4	Immediate					
	1 minute	Suspicious (brown)	Suspicious (brown)	Negative	Too dark to test	Too dark to test
	1 minute	Suspicious (brown)	Suspicious (brown)	Negative	Too dark to test	Too dark to test
	5 minutes	Suspicious (brown)	Suspicious (brown)	Negative	Too dark to test	Too dark to test

TABLE 2.—Concluded.

*Aniline chloride test.*

(Fodor's.)

SERIES B—½ HOUR AT 180°F.

SAM- PLE	TIME	POWERS	WRIGHT	KATZ	SEAMAN	SHERWOOD
1	Immediate	Too dark to test	Negative	Negative (slight pink tint)	Too dark to test	Too dark to test
	1 minute	Too dark to test	Suspicious (pinkish)	Negative	Too dark to test	Too dark to test
	5 minutes	Too dark to test	Negative (brown)	Negative	Too dark to test	Too dark to test
2	Immediate	Suspicious	Negative	Suspicious (slight pink)	Negative (pinkish yellow)	Suspicious
	1 minute	Positive (light pink)	Negative (pinkish)	Negative (weaker)	Suspicious (deep pink)	Positive
	5 minutes	Positive (light pink)	Negative (brownish pink)	Negative	Positive (light amber)	Positive
3	Immediate	Positive	Negative	Suspicious (strong pink)	Too dark to test	Too dark to test
	1 minute	Positive (cherry red)	Suspicious (pinkish)	Negative (weaker)	Too dark to test	Too dark to test
	5 minutes	Suspicious (brown)	Suspicious (pinkish)	Negative	Too dark to test	Too dark to test
4	Immediate	Too dark to test	Negative	Negative	Too dark to test	Too dark to test
	1 minute	Too dark to test	Negative (slight pinkish)	Negative	Too dark to test	Too dark to test
	5 minutes	Too dark to test	Negative (brown)	Negative	Too dark to test	Too dark to test

SERIES C—20 MINUTES AT 208°-209°F.

1	Immediate	Too dark to test	Suspicious (slight pink)	Suspicious (pink)	Too dark to test	Too dark to test
	1 minute	Too dark to test	Suspicious (pinkish)	Negative (much weaker)	Too dark to test	Too dark to test
	5 minutes	Too dark to test	Negative (brown)	Negative	Too dark to test	Too dark to test
2	Immediate	Negative	Negative	Suspicious (pink)	Negative (yellow)	Suspicious
	1 minute	Positive (light pink)	Negative (pinkish)	Negative (much weaker)	Suspicious (deep pink)	Positive
	5 minutes	Positive (light pink)	Negative (brownish pink)	Negative	Positive (medium dark amber)	Positive
3	Immediate	Positive	Negative	Positive (cherry red)	Too dark to test	Too dark to test
	1 minute	Positive (cherry red)	Positive (red)	Suspicious (much weaker)	Too dark to test	Too dark to test
	5 minutes	Suspicious (brown)	Suspicious (reddish brown)	Negative	Too dark to test	Too dark to test
4	Immediate	Too dark to test	Negative	Negative	Too dark to test	Too dark to test
	1 minute	Too dark to test	Negative (brown)	Negative	Too dark to test	Too dark to test
	5 minutes	Too dark to test	Negative (brown)	Negative	Too dark to test	Too dark to test



## REPORT ON MAPLE PRODUCTS.

By H. M. LANCASTER (Department of Health, Ottawa, Can.), *Associate Referee*.

The following details for the determination of the lead number are the result of a development covering a period of several years. The fundamental idea was to estimate the amount of lead thrown down from a basic acetate solution, with a view to obtaining information as to whether or not samples submitted were genuine maple sirups or were adulterated.

## THE PREPARATION OF BASIC LEAD ACETATE SOLUTION.

(1) *Prepared from lead acetate and litharge*.—Into a one-liter evaporating dish weigh 215 grams of normal lead acetate crystals and 65 grams of litharge. Add about 500 cc. of water. Heat to boiling and boil exactly 30 minutes. Allow to cool in the dish or pour off into a Pyrex beaker. Take the specific gravity at 20°C. and dilute with recently boiled distilled water to a density of 1.25.

(2) *Prepared from Horne's salt*.—Boil 280 grams of Horne's dry basic lead acetate with 500 cc. of water. When solution is complete except for a slight sediment, allow to cool in the dish or pour off into a Pyrex beaker, and dilute to a density of 1.25 at 20°C. as in (1).

## DETERMINATION.

Weigh the quantity of sirup containing 25 grams of dry matter and transfer to a 250 cc. beaker with distilled water; boil, transfer to a 100 cc. volumetric flask, cool to room temperature, and make up to 100 cc. with distilled water. Pipet 20 cc. into a large test tube. Add 2 cc. of the basic lead acetate reagent, mix, allow to stand 2 hours, filter on a tared Gooch crucible having a mat at least 3 mm. thick, wash four or five times with boiling water, dry at 100°C., and weigh. Multiply the weight of the dry precipitate in grams by 20.

The basic lead acetate solution is not always of exactly the same composition even when prepared by the same analyst. With a view to ascertaining what effect this discrepancy might have upon the lead numbers obtained, a sample of sirup was analyzed, the basic lead acetate reagents prepared in four different laboratories being used for this purpose. A summary of the results obtained is shown in Table 1.

TABLE 1.

*Lead number of sirup sample, different basic acetate solutions being used. Analyst, M. E. Whalley.*

<i>Reagent prepared in—</i>	<i>pH</i>	
Ottawa.....	(7.5)	1.25—1.20—1.21
Winnipeg.....	(7.7)	1.36—1.29—1.30
Montreal.....	(7.5)	1.25—1.33—1.26
Halifax.....	(7.35)	1.23—1.35—1.14
		1.14—1.27
Vancouver.....	(7.3)	1.06—1.08—1.03

The hydrogen ion concentrations were determined colorimetrically, phenol red being used.

These results indicate that the hydrogen ion concentration of the reagent may vary between limits indicated by pH 7.3 and pH 7.7. If the

reagent is more strongly alkaline, the lead number is inclined to be higher, although the result 1.35, obtained by using the reagent from the Halifax laboratory and shown in Table 1, is anomalous from this standpoint. In fact, later work shows that variations in technique have a greater influence upon results than has the reaction of the reagent.

In order to extend the investigation, the same sample of sirup was submitted to eight different analysts with instructions to use—

- (1) Basic lead acetate solution supplied from Ottawa laboratories, and
- (2) Basic lead acetate solution prepared locally.

The summary of the results obtained is given in Table 2.

TABLE 2.

*Results of determination of Canadian lead number with different reagents.*

ANALYST	SAMPLE NO 8677*		SAMPLE NO 8676		SAMPLE NO 8675	
	Reagent prepared in Ottawa	Reagent prepared locally	Reagent prepared in Ottawa	Reagent prepared locally	Reagent prepared in Ottawa	Reagent prepared locally
A. Valin, Montreal	1.22	1.44	. . .	. . . .	4.46	4.98
	1.22	1.52			4.54	4.96
	1.22	1.48			4.50	5.00
						4.98
A. P. Couture, Montreal	1.33	1.50	. . .	.	4.64	4.95
	1.27	1.53			4.64	4.96
	1.30	1.52			4.64	4.96
C. C. Forward, Halifax	1.34	1.31			4.54	4.49
	1.31	1.24			4.61	4.43
	1.28	1.29			4.57	4.52
M. E. Whalley, Ottawa	1.58		3.57		5.0	
	1.56		3.51		5.09	
	1.59		3.54		5.04	
H. A. Watson, Winnipeg	1.28	1.47	3.62	3.70		
	1.37	1.45	3.67	3.79		
E. L. C. Forster, Winnipeg	1.36	1.24	3.24	3.60		
	1.34	1.32	3.36	3.48		
W. A. Davidson, Vancouver	1.34	1.28	3.26	3.20		
	1.36	1.30	3.28	3.18		
	1.46	1.34	3.38	3.20		
	1.40		3.40	. . .	. . .	. . . .
L. E. Johnson, Ottawa	1.54		3.78			
	1.59	. . .	3.74	. . .	. . .	. .
	1.59		3.76		. . .	
Maximum	1.59		3.79		5.09	
Minimum	1.22		3.18		4.43	
Average	1.38		3.48		4.75	

\* This sample was not genuine maple sirup, and in no case did the analyst obtain a figure that would indicate otherwise. A similar procedure was carried out with two other samples, which were genuine maple sirups.

The technique, as it stands, is sufficiently accurate to supply figures that are a valuable index as to the genuineness of maple products. These figures are by no means all-sufficient, and they might be made more useful in border-line cases if the error inherent in the method could be reduced. At present it appears that the importance of slight differences in the reagent has been somewhat exaggerated and that the washing of the precipitate is the step in the analytical process that requires standardizing.

However, it must be borne in mind that the analyses of several hundred genuine Canadian maple sirups have shown that the lead numbers range between wide limits (2.25–5.5). It can not be expected, therefore, that this analytical method (or any other method based on precipitation from basic lead acetate) can be improved to the point at which it will indicate the adulteration of certain types of maple sirup with as much as 40 per cent of cane sugar sirup.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that this method be subjected to further study and compared with the Winton method as to accuracy obtainable and the time required.

#### REPORT ON MALTOSE PRODUCTS.

By F. W. REYNOLDS (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The work this year has been confined to an effort to formulate an enzyme method for the determination of maltose in the presence of other polysaccharides and to obtain an active stable maltase preparation to be used as an analytical reagent. Experiments in concentrating the enzyme by ultrafiltration are encouraging, but serious difficulties are met in obtaining a reasonably active crude maltase preparation by extraction from yeast and in stabilizing this preparation sufficiently to make it suitable as an analytical reagent. So far results in this direction on the part of the associate referee have been unsatisfactory, but current correspondence with a group of men who are working on this problem in one of the colleges may prove beneficial. Advantage will be taken of their results as soon as they are available, and it is hoped that substantial progress along these lines will be made within the next year.

There is a great demand in the corn sirup industry for a reliable method for maltose determination, and a method, if developed, will be of substantial practical value to this industry, as well as to the A. O. A. C. It is recommended, therefore, that this work be continued<sup>1</sup>.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 260.

## REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS.

By J. F. BREWSTER (Louisiana Sugar Experiment Station, New Orleans, La.), *Associate Referee*.

The present report covers a continuation of the collaborative work upon moisture and solids in sugar-house products and may be regarded as supplementary to the similar report published last year<sup>1</sup>. The materials distributed for collaborative analysis were one sample each of raw cane sugar, beet molasses, pure cane sirup (table sirup), medium cane molasses, and a synthetic sirup of known solid content somewhat resembling a table sirup but containing more invert sugar than is usual in the latter.

The thanks of the associate referee and the association are due the following collaborators, who reported results:

R. T. Balch, Carbohydrate Laboratory, Bureau of Chemistry, Washington, D. C.

S. M. Byall, Penick and Ford, Ltd., New Orleans, La.

Earl Durfee, Continental Sugar Co., Toledo, Ohio.

F. C. Hargreaves, Amalgamated Sugar Co., Ogden, Utah.

Theo. Markovits, Penick and Ford, Ltd., New Orleans, La.

I. W. Reed, American Beet Sugar Co., Rocky Ford, Colo.

A. Seidenberg, Chemical Laboratory, Dept. of Health, New York, N. Y.

R. J. Smith, Holly Sugar Corporation, Colorado Springs, Colo.

W. Stimmel, Utah-Idaho Sugar Co., Salt Lake City, Utah.

J. H. Zisch, Great Western Sugar Co., Bayard, Nebr.

F. C. Zitkowski, American Beet Sugar Co., Oxnard, Calif.

For the determination of moisture in the raw sugar sample results were reported upon the following procedures:

1. The official method, heating 10 hours in a boiling water oven and weighing, then repeating the heating for periods of 1 hour until there is only a slight change in weight.

2. Heating to constant weight at 70°C. with reduced pressure.

3. Heating to constant weight at 100°-105°C.

4. Drying by means of the Spencer oven.

5. Heating to constant weight at 105°-110°C.

6. Heating 2 hours at 98°C. followed by further heating 1 hour at 105°C. and weighing.

7. Heating 3 hours at 98°C. and weighing.

8. Drying to constant weight at 100°C. and with reduced pressure.

The results of the analysis of the raw sugar are shown in Table 1, in which the methods are numbered as above. Only averages of the individual analysts are shown.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 354.

TABLE 1.  
Raw cane sugar, percentage of moisture.

ANALYST	METHOD							
	1	2	3	4	5	6	7	8
Balch .....	1.82 1.90	1.60 1.62	2.04 2.09	1.65 1.59				
Average.....	1.86	1.61	2.06	1.62				
Durfee Finlay Laboratory Blissfield Laboratory			2.03 1.80					
Average.....			1.96					
Hargreaves ..... "L" "C"	1.49*				1.75* 1.35†	1.73* 1.84	1.76	
Average.....	1.49					1.78	1.76	
Reed .....	1.76	1.74			1.99			2.02
Smith.....							1.23	
Stimmel.....	1.81	1.54				1.16† 1.87		
Zisch .....	1.75	1.34		1.66-110° 1.58-105°		1.96		
Average.....	1.75	1.34		1.62		1.96		
Zitkowski .....	1.81	1.82			1.90			2.10
General average...	1.75	1.61	1.99	1.62	1.88	1.85		2.06

\* Sample crushed for analysis.

† Omitted from averages.

## DISCUSSION OF RESULTS.

The true moisture content of the raw sugar is unknown, and there is considerable variation between extreme results for single methods excepting the Spencer oven, so that a consideration of averages is perhaps the only safe method of approach. In drying material such as raw sugar the same difficulties are confronted as in the drying of molasses, for the sugar crystals are inclosed in a molasses film. If temperatures are too low, there is the risk of not evaporating all the moisture. If the drying temperature is too high or the period of heating too protracted, there is danger of decomposition. The results throughout show greater losses at the higher temperatures and longer heating periods, whereas at high temperature and an arbitrary short period of heating, the results more closely approach a mean. The mean of the general average for all methods is 1.82 per cent. The closest approach to this is 1.85 per

cent by Method 6, but only three analysts are represented and one result was omitted from the averages. Method 1, the official method, containing six sets of results, is perhaps more acceptable. If one low result is left out here the average for this method becomes 1.80 per cent. It has been pointed out by Zisch that at Bayard, Nebr., water boils at 95°C. This is a difference of 5° between that point and Washington or New Orleans. It is likely that the difference at Colorado Springs is even greater. It has already been recommended that a definite specification of temperature for this method be stated. As a matter of fact the temperature inside a boiling water oven at sea level (New Orleans) is about 95°C. This is perhaps the temperature preferable for such a long heating period.

#### SIRUPS AND MOLASSES.

Results for the determination of solids in the liquid products by the following methods were reported:

1. The pumice method at 70°C. and reduced pressure.
2. The official sand method.
3. The official sand method at 70°C. and reduced pressure.
4. Sand as distributing medium with Aikin's<sup>1</sup> modifications (100°-105°C.).
5. Seidenberg's gauze dish, temperature 50°-60°C. and atmospheric pressure. The directions for the determination of solids in sugar-house products have been published<sup>2</sup>.
6. Seidenberg's gauze dish with Seidenberg's directions, employing a temperature of 70°C. and reduced pressure, 5 inches mercury, absolute, or less.
7. Drying by means of the Spencer oven following suggestions of Balch<sup>3</sup>.
8. Densimetric methods.
9. Refractometric methods.

The results of solids determinations are given in Tables 2-5, and the numbers heading the columns under "Method" correspond to those in the above list of methods. Table 6 is a table of averages arranged to facilitate comparison.

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<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 979.

<sup>2</sup> *Ibid.*, 737; *J. Assoc. Official Agr. Chemists*, 1923, 7: 98.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 357.

TABLE 2.  
*Beet molasses, percentage of solids.*

ANALYST	METHOD								
	1	2	3	4	5	6	7	8	9
Balch .....	78.42	78.82	78.18	78.32	81.35	79.85	78.86	85.50	
	78.53	78.74	78.80	78.20	81.00	80.10	78.76	85.40	
	78.57	78.77	78.97	78.26	80.82	80.20	78.80		
	78.49	78.71	78.70	78.39	80.76	79.25	78.62	85.46	
	78.44	78.64	78.80	78.49			78.68	85.38	
	78.52	78.59	78.84	78.50			78.78		77.12
Average.....	78.50	78.71	78.91	78.36	80.98	79.85	78.75	85.43	77.12
Durfee .....				78.50					78.40
				78.47					78.0
Average.....				78.48					78.2
Hargreaves .....	78.56	dilute d 1:1	77.75	dilute d 1:1					
	78.61	dilute d 1:3	77.87	dilute d 1:3					
Average.....	78.58		77.81						
Reed .....				78.97				83.80	79.20
				78.87					
Average.....				78.92				83.80	79.20
Smith.....				78.52	dilute d 1:1				
				78.40	dilute d 1:1				
				78.54	original molasses				
				78.58	original molasses				
Average.....				78.51					
Stimmel.....	79.28	78.57	79.63	78.23	80.88	80.02			77.95
	79.19	78.61	79.55	78.22	80.75	79.98			
	79.27	78.51	79.50	78.21	80.96	80.00			
	79.14	78.68	79.46	78.20	80.86	80.01			
	79.29	78.49	79.56	78.21	80.74	80.06			
	79.32	78.44	79.59	78.25					
	79.23	78.50							
Average.....	79.25	78.54	79.55	78.22	80.84	80.01			77.95
Zisch .....	79.93	78.49	79.20	78.41	79.82	79.88	78.58	85.26	
	79.80	78.47	79.20	78.27	79.87	79.86	78.71	85.34	
Average.....	79.86	78.48	79.20	78.34	79.84	79.87	78.64	85.30	
Zitkowski .....		79.09	78.97	78.97				83.90	79.10
General average*..	79.05	78.70	79.16	78.45	80.55	79.91	78.69	84.61	78.31

\* Computed from individual averages.

TABLE 3.  
*Synthetic sirup, percentage of solids.*

ANALYST	METHOD								
	1	2	3	4	5	6	7	8	9
Balch .....	69.41 69.45 69.38	69.64 69.63 69.66	69.58 69.59 69.55	69.55 69.46 69.44	71.34 71.36	70.17 70.05	69.72 69.69 69.70	70.40 70.28	68.79
Average.....	69.41	69.64	69.57	69.48	71.35	70.11	69.70	70.34	68.79
Byall .....	69.56 69.65		71.90 71.70					70.20	69.00
Average.....	69.60		71.80					70.20	69.00
Durfee .....				69.70 69.75					
Average.....				69.72					
Hargreaves .....	69.71			69.01 68.95	sample undiluted	1:1			
Average.....	69.71			68.98					
Markovits.....	70.09 70.02		71.32 71.58					69.80	69.60
Average .....	70.05		71.45					69.80	69.60
Reed .....		69.79	69.84	69.60	105 to 110°			69.65	69.75
Seidenberg .....	70.58 70.45				71.43 72.02 71.83 71.78 71.71 71.69	55°-7 48°-7 48 to 54°-7 58°-7	60 m 60 m 60 m 60 m	m. m. 0 mm. m. m.	
Average.....	70.51				71.74				
Smith.....				69.34 69.68 69.62 69.67	diluted 1:1 diluted 1:1 sample undiluted sample undiluted				
Average.....				69.58					
Stimmel.....	70.09 70.05 70.19 70.33 70.02 69.95	69.47 69.60 69.58 69.46 69.64 69.41 69.43 69.54 69.47	70.23 70.17 70.14 70.17 70.23 70.02 70.29 70.36 70.04	69.20 69.10 69.12 69.14	71.67 71.74 71.88 71.86	70.67 70.45 70.50 70.40			69.38
Average.....	70.11	69.51	70.18	69.14	71.79	70.51			69.38
Zisch .....	70.34 70.58	69.41 69.40	69.14 69.14	68.87 68.89	69.68	69.60	69.48	70.17 70.11	
Average.....	70.47	69.40	69.14	68.88	69.68	69.60	69.48	70.14	
Zitkowski .....		69.84	69.85	69.63				69.60	69.70
General average* ..	69.99	69.64	70.26	69.37	71.14	70.07	69.59	69.95	69.37

\* Calculated from individual averages.



## PREPARATION OF SYNTHETIC SIRUP.

A tared flask received 475 grams of confectioners' crystals, which were dissolved and inverted in standard sulfuric acid by gentle warming. After complete inversion the acid was exactly neutralized with standard sodium hydroxide solution. Cane gum was dispersed in standard sodium hydroxide and exactly neutralized with standard sulfuric acid, then added to the invert sirup. More confectioners' crystals were added to the neutral mixture with enough water to furnish a sirup approximating 70 per cent solids, and the whole was warmed to dissolve the sugar. The use of the tared flask is obvious since it is necessary to know the weights of solids added and the weight of finished sirup. Besides a means is thus provided for checking the ingredients that had been weighed separately.

Solids are calculated on a dry basis in the table that follows from determinations at 100°C. and 5 mm. mercury over phosphorus pentoxide. Sodium sulfate was calculated from the volume of standard acid and alkali used in the preparation.

The same general procedure was used in the preparation of the 1923 sample of synthetic sirup.

TABLE 4.  
Pure cane sirup, percentage of solids.

ANALYST	METHOD								
	1	2	3	4	5	6	7	8	9
Balch .....	71.45	71.41	71.47	71.29	73.55	72.29	71.81	73.70	
	71.44	71.33	71.65	71.07	73.55	72.34	71.85	Brix	
	71.45	71.47	71.61	71.08	73.68	72.51	71.84	Spindle	
	71.42	71.46	71.65	71.31	73.61	72.24	71.80	73.62	
	71.36	71.42	71.68	71.31			71.74	Pycno	meter
		71.46	71.62	71.41			71.90		71.32
Average.....	71.42	71.43	71.61	71.25	73.60	72.35	71.82	73.66	71.32
Byall .....	72.10		72.92		82.45*			73.20	71.30
	72.40		73.25		82.54*			Pycno	meter
Average.....	72.25		73.08		82.49*			73.20	71.30
Markovits.....	72.04		73.50					73.40	71.90
	72.53		72.92					Pycno	meter
Average.....	72.26		73.21					73.40	71.90
Seidenberg .....	72.96				73.00	72.80			
	71.83				72.96	72.97			
	73.05				72.58	72.97			
Average.....	72.61				72.85	72.91			
Zisch .....	72.15†		72.42†		74.62	72.91	72.27		
	72.16		72.39		74.47	73.00	72.13		
Average.....	72.15		72.40		74.54	72.95	72.20		
	72.47§		72.48§						
	72.52		72.45						
Average.....	72.49		72.46						
General average†..	72.19	71.43	72.55	71.25	73.66	72.74	72.01	73.42	71.51

\* Omitted from general average.

† Calculated from individual averages.

‡ Corrected for retained moisture in parallel wetted blanks.

§ Uncorrected.

## COMPOSITION OF SIRUP.

	<i>grams</i>
Sucrose.....	1061.00
Invert sugar.....	500.00
Cane gum.....	24.05
Sodium sulfate.....	13.55
Total solids.....	1598.60
Water....	697.40
Total sirup.....	2296.00
Percentage of solids .....	69.62

TABLE 5.

*Medium cane molasses, percentage of solids.*

ANALYST	METHOD								
	1	2	3	4	5	6	7	8	9
Balch .....	74.52	74.31	74.77	73.36	76.44	75.36	74.83	80.10	74.40
	74.38	74.23	74.85	73.01*	76.48	75.59	74.84	80.05	
	74.38	74.25	74.85	73.25	76.35	75.25	74.89		
	74.70	74.33	75.02	73.39	76.63	75.15	74.75		
	74.72	74.25	75.13	73.25			74.89		
	74.68	74.39	75.42*	73.28			74.94		
Average. ....	74.56	74.29	74.92	73.31	76.48	75.34	74.86	80.07	74.40
Byall .....	76.40		76.12					79.10	75.30
	76.38		76.90						
Average. ....	76.39		76.51					79.10	75.30
Markovits. ....	75.71		76.11					79.60	75.70
	75.80		76.62						
Average.....	75.75		76.36					79.60	75.70
Seidenberg .....	76.39				76.75	76.73			
	76.44				76.41	76.32			
	75.96				76.61	76.58			
	76.26				76.59	76.54			
Zisch† .....	75.87		75.33		77.16	75.71	74.76		
	75.55		75.38		77.04	75.87	75.03		
Average....	75.71		75.35		77.10	75.79	74.89		
Zisch*† .....	75.36		75.26						
	75.09		75.31						
Average.....	75.22		75.28						
General average...	75.73	74.29	75.78	73.31	76.72	75.89	74.87	79.71	75.13

\* Omitted from averages.

† Corrected for retained moisture in parallel wetted blanks.

‡ Uncorrected.

TABLE 6.  
Comparison of averages.

METHOD	BET MOLASSES	SYNTHETIC SIRUP	CANE SIRUP	CANE MOLASSES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. Pumice, 70°C.—Vacuum.....	79.05	69.99	72.19	75.73
2. Sand, Water Oven.....	78.70	69.64	71.43	74.29
3. Sand, 70°C.—Vacuum.....	79.16	70.26	72.55	75.78
4. Sand, 100°–105°C.....	78.45	69.37	71.25	73.31
5. Gauze, 50°–60°C.....	80.55*	71.14*	73.66*	76.72*
6. Gauze, 70°C.—Vacuum.....	79.91	70.07	72.74	75.89
7. Spencer Oven.....	78.69	69.59	72.01	74.87
Mean.....	78.99	69.82	72.03	74.98
8. Densimetric.....	84.61	69.85	73.43	79.71
9. Refractometric.....	78.31	69.37	71.51	75.13
Mean.....	81.46	69.61	72.47	77.42

\* Omitted from mean.

#### DISCUSSION OF RESULTS.

A survey of the tables shows a disagreement among methods as well as disagreement among analysts for any given method. The exact determination of moisture or solids in sirups and molasses is not an easy matter, and reasons therefor have often been given. The densimetric and refractometric methods may be expected to furnish acceptable results only when applied to the determination of solids in high-grade products, that is, in products from which no great quantity of sugars has been withdrawn. The withdrawal of sugar leaves the impurities more highly concentrated, so that little accuracy may be expected of these methods in grades lower than juices, virgin sirups (thick juice), or remelt sugars. The application of the drying methods is beset with other difficulties. Temperatures must be high enough to insure the driving off of all the water, whether it be water of solution, water of crystallization or water physico-chemically held back by colloids or mechanically by the formation of films. On the other hand, too high temperatures lead to decomposition of fructose when present, but perhaps more seriously to the decomposition of other organic solids present in low-grade products, such as molasses, that have already been subjected repeatedly to the action of heat.

The results and averages fall roughly into two groups. The higher figures were obtained at the lower temperatures as in Methods 1, 3, 5, and 6, while the lower group was obtained at higher temperatures by Methods 2, 4, and 7. The same observation was made last year.

Table 3, showing results for the drying of the synthetic sirup of known solid content, is of especial interest. Method 2 yielded an average of 69.64 per cent solids, the calculated being 69.62 per cent. The extremes

of individual determinations are 69.84 and 69.40 per cent, respectively, a difference of 0.44, the lowest difference of this table. The next nearest approach to the calculated solid content is furnished by the results obtained by the Spencer oven. The average of results by Method 4 is fair, but the difference between extremes is double that of Method 2. The results from Methods 2 and 4 are contradictory to the belief that mixtures containing fructose should not be heated to temperatures above 70°C. It can not be assumed that a part of the loss in weight is due to decomposition and that the closeness of the figures to the calculated is accidental. It is demonstrated, however, when comparison is made with the vacuum methods, 1, 3, and 6, that a relatively high temperature is necessary to drive off the moisture. This temperature should then be between 90 and 95°C. The synthetic sirup is simple in composition as compared with natural products such as the pure cane sirup and molasses samples. It is perhaps more nearly related to the cane sirup, and by analogy it would be reasoned that methods giving good results with the synthetic sirup are the proper methods to be employed in the analysis of cane sirup. Only one analyst reported results upon the cane sirup by Methods 2 and 4, Table 4, so that a conclusion therefrom would seem unwarranted.

In the analysis of beet and cane molasses, Tables 2 and 5, the same trend of results is to be noted, depending upon the temperatures of drying. Method 4 is used almost exclusively by the beet sugar chemists. In the averages for Methods 2 and 4 the difference is not extraordinary. The Spencer oven results approximate these closely. Last year there was close agreement between the official sand method and pumice at 70°C.

As usual, cane molasses seems to offer the greatest difficulty where concordant results are sought. This material contains so much that is readily decomposable that the employment of comparatively low temperatures seems imperative. As regards the distributing medium, it appears to make little difference whether sand or pumice is used. The sand, being easier to prepare, will naturally be preferred. In this connection it should be pointed out that the gauze dish at 70°C. and reduced pressure gave results in line with the other vacuum methods. The temperatures of 50°-60°C. at atmospheric pressure are obviously too low to dry sirups or molasses thoroughly within a reasonable length of time. The results reported where the latter conditions obtained, with one exception, were extremely high. This holds for the analysis of all the products.

It would appear that up to the present none of the methods generally used for the determination of solids by drying is to be regarded as uniformly reliable. As pointed out previously, sirups and molasses are not easy of analysis, and more research upon the determination of moisture is necessary.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the present official method for the determination of moisture in sugars be unchanged, except that in the text it be specified that the temperature be maintained at 100°C.

(2) That low-grade sugars be included in the collaborative work in the coming year.

(3) That the recommendations made last year regarding the official pumice and sand methods be effective this year

(4) That in view of the fact that methods for the drying of sirups and molasses are becoming very numerous without apparently giving greater reliability the collaborative work of the coming year be directed toward an effort to diminish the number of methods and that trial be given to the procedure similar to the Brown-Duvel<sup>2</sup> method recently worked out by Bidwell<sup>3</sup>.

(5) That collaborative work on the determination of solids be continued.

## REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*.

The polariscopic methods given in the last edition of the *Book of Methods*<sup>4</sup> of the association were formulated in accordance with the recommendations of the referee on sugar for the year 1915. Since that time no change in these methods has been made other than that suggested by the associate referee on sugar for 1919 in regard to temperature corrections to be applied to the polarization of raw cane sugars.

At the seventh session of the International Commission for Uniform Methods of Sugar Analysis, held in New York City in 1912, a committee was appointed to redetermine the value of the Clerget divisor, and the recommendations of the referee on sugar for 1915 were the outcome of the latter's studies undertaken up to that time.

During the past ten years or so, various investigators have made important contributions to this subject—notably Browne, Jackson and Gillis, Sazavsky, Schrefeld, Stanek, and Steuerwald. The present associate referee was requested, therefore, to carry out such investigations as seemed advisable to bring the polariscopic methods of the association, so far as possible, up to date. As it was decided to confine the work for this year to products free from raffinose, the results obtained by the

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 260.

<sup>2</sup> U. S. Dept. Agr. Bull. 56, Bur. Plant Industry Circ. 72.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 295.

<sup>4</sup> *Assoc. Official Agr. Chemists, Methods*, 1920.

associate referee apply to materials of this character only, and not to beet products. Most of the experimental work was done by R. T. Balch, of the Bureau of Chemistry, and by Messrs. C. A. Gamble and G. H. Hardin, of the New York Sugar Trade Laboratory. The writer gratefully acknowledges their cooperation.

The first of the two official methods for the determination of sucrose at present in use by the association is that of acid hydrolysis; it is based on Herzfeld's<sup>1</sup> procedure, and the general factor 142.66 is used. This factor has been retested by a number of investigators, and constants as low as 142.65 and as high as 143.09 have been reported. The reason for this discrepancy appears to be the fact, pointed out especially by Jackson and Gillis, that at the high temperature used for inversion, slight variations in manipulation may cause a decided change in the factor, as inversion and reversion may occur simultaneously. In any method of acid hydrolysis requiring high temperatures these conditions will become even more aggravated if invert sugar is present in the original product; here reversion of the invert sugar may begin before the inversion of the sucrose has proceeded very far.

Nevertheless, since the plain acid method of hydrolysis is very widely used on account of its simplicity, and since in many products the presence of free acid of itself does not lead to errors, it will be wise to retain it until other methods proposed more recently have been given a thorough trial. This necessitates, however, the elimination of known sources of error so far as is possible.

#### PREVIOUS INVESTIGATIONS.

Steuerwald<sup>2</sup> in a careful investigation of Herzfeld's method found an average factor of 143.05. Stanek<sup>3</sup>, who obtained 142.93, proposed the adoption of the round average value 143.0. Jackson and Gillis<sup>4</sup> computed from their results a figure between 143.06 and 143.11. Schrefeld<sup>5</sup> used a more carefully controlled method of heating, which he described in full detail, and this method yielded the value 143.0. The latter figure has been confirmed by Browne<sup>6</sup>. In order to check these values once more, nine determinations were made with Domino tablet sugar, the very explicit directions given by Schrefeld being followed most carefully. These tests resulted in an average value of  $142.97 \pm 0.02$ . The probable mean error of a single determination was  $\pm 0.065$ .

The average of the inversion factors found in the five independent investigations cited is 142.99, which may well be rounded off to 143.0, as proposed by both Stanek and Schrefeld. The latter's procedure of

<sup>1</sup> *Z. Ver. deut. Zucker-Ind.*, 1888, 38: 699.

<sup>2</sup> *Arch. Zuckerind.*, 1913, 21: 1383.

<sup>3</sup> *Z. Zuckerind.*, Böhmen, 1914, 38: 298.

<sup>4</sup> U. S. Bur. Standards Sci. Paper 375, 153.

<sup>5</sup> *Z. Ver. deut. Zucker-Ind.*, 1920, 70: 402.

<sup>6</sup> *J. Ind. Eng. Chem.*, 1921, 13: 794.

inversion, allowing very little latitude in detail of manipulation, commends itself for adoption.

For the second of the official methods of sucrose determination Browne<sup>1</sup> has established the general factor 142.0, which is official at the present time. His results have been confirmed independently by Jackson and Gillis<sup>2</sup> and more recently by Sazavsky<sup>3</sup>.

A few months ago the Bureau of Chemistry<sup>4</sup> reported that the purer and more concentrated preparations of invertase made by ultrafiltration give a somewhat higher value than 142.0, and the writer<sup>5</sup> has, on the basis of Vosburgh's<sup>6</sup> polarimetric measurements on invert sugar, also reached the conclusion that 142.0 seems somewhat too low.

Eight measurements of this constant were made, Domino tablet sugar being used as before. The invertase, of  $k = 0.1$ , was kindly furnished by the Carbohydrate Laboratory of the Bureau of Chemistry. The inversions were made at 26°–27°C. overnight, but the final polarizations were made as late as 24 hours after the beginning of the test to allow completion of mutarotation. In these eight tests an average value of  $142.10 \pm 0.025$  was obtained, the mean probable error of a single determination being  $\pm 0.07$ .

The results of the Bureau of Chemistry have thus been confirmed experimentally also. However, in the judgment of the writer, it would be premature to suggest a change in the adopted factor until it is definitely known whether or not the factor changes with the purity and concentration of the enzyme solution used.

The third principal object of the associate referee's work for the present year was to decide, if possible, two questions of fundamental importance in sugar analysis by polarimetric methods, viz:

(1) Whether in the analysis of sugar mixtures the change in the Clerget divisor with change in concentration should be based on the difference between the direct and invert polarizations, in other words on the sucrose concentration, or on the total sugar concentration.

The literature on this subject will not be taken up *in extenso*, but it may be pointed out that Vosburgh<sup>6</sup>, confirming former work by Rosanoff<sup>7</sup>, found that the specific rotation of sugar mixtures is not determined by the specific rotation of the components at their partial concentration, but by that at the total sugar concentration. Browne<sup>8</sup> called attention to the important applications of this rule to practical sugar analysis.

<sup>1</sup> J. Assoc. Official Agr. Chem., 1916, 2: 140.

<sup>2</sup> U. S. Bur. Standards Sci. Paper 375, 167.

<sup>3</sup> Z. Zucker-Ind. czechoslovak Rep., 1924, 48: 255.

<sup>4</sup> R. T. Balch. The determination of sucrose and raffinose in beet products by the enzyme method. Paper presented at the Washington meeting of the American Chemical Society, April, 1924. Published in Ind. Eng. Chem., 1925, 17: 240, under the title "Application of Enzymes to Beet Sugar Factory Control".

<sup>5</sup> J. Am. Chem. Soc., 1925, 46: 1104.

<sup>6</sup> Ibid., 1921, 43: 219.

<sup>7</sup> Ibid., 1911, 33: 1911.

<sup>8</sup> Louisiana Planter, 1921, 67. 44.

(2) Whether or not the methods of neutral polarization proposed by Saillard, and worked out in detail by Jackson and Gillis, give results that check with the invertase method.

The work for the present year was planned in such a way that it might be expected to furnish an answer to both of the above questions. This made it necessary to avoid the complications that are liable to arise when products already containing invert sugar are heated to high temperatures in the inversion process. It was decided, therefore, to carry out the inversion in every case by allowing the solutions to stand overnight at a temperature of  $26^{\circ}$ – $27^{\circ}$ C., which is about the average between that of a heated room in the winter and room temperature in midsummer. According to Jackson and Gillis, standing overnight is sufficient to complete inversion by acid under the conditions prescribed by them; it is also sufficient for complete inversion, by means of 10 cc. per 100 cc. of solution, of the ultrafiltered invertase mentioned above; this fact was established by a number of preliminary tests. The final readings were postponed, generally, until about 24 hours after the beginning of the tests in order to attain constant rotation without resorting to the use of heat or alkali, either one of which might affect the rotation of the invert sugar.

Another important precaution taken in this work was to have the solutions for both direct and invert polarizations at the same concentration. This necessity was recognized over a generation ago by Gubbe<sup>1</sup> and by Landolt<sup>2</sup>, and was again pointed out by Jackson and Gillis. Browne, likewise, had the same idea in mind when he recommended a return to Clerget's original procedure of diluting to one-tenth greater volume instead of doubling the volume for the invert polarization.

It was originally planned to use the three following raw materials in this investigation:

- (1) Sucrose (Domino tablet sugar);
- (2) Invert sugar, in the form of a sirup prepared from sucrose by means of invertase; and
- (3) A sirup containing all the non-sucrose impurities of a cane molasses, prepared from a blackstrap by inversion with invertase, clarified with lead subacetate, and de-leaded with dry potassium oxalate.

Sucrose was then to be determined in all these three products, as well as in mixtures of 1 and 2, and of 1 and 3, containing three different proportions of sucrose and non-sucrose solids, viz., 3 to 1, 1 to 1, and 1 to 3.

On account of lack of time, it was not possible, in either of the two laboratories in which this work was performed, to include the non-sucrose sirup from molasses; therefore the work was confined to sucrose, invert sugar sirup, and mixtures of these two.

<sup>1</sup> *Ber.*, 1885, 18: 2207.

<sup>2</sup> *Ibid.*, 1888, 21: 212.



## METHODS STUDIED.

The modifications of the Clerget method selected for study were:

(1) The official acid method, inversion being carried out at a temperature of 26°–27°C. overnight. In the calculation of the results the basic factor 143.2 was used; this is the rounded-off average of Schrefeld's results (143.2), Jackson and Gillis' results (143.25), and of four tests in this laboratory (143.18).

(2) The official invertase method, with 10 cc. ultra-filtered invertase of  $k = 0.1$ , at 26°–27°C. overnight. The basic factor used was 142.0.

(3) Jackson and Gillis method No. II, in which ammonium chloride is added to the solution for direct polarization, and the acid in the invert solution neutralized with ammonia. The basic factor found for this method by Jackson and Gillis is 143.34. The inversion was carried out at 26°–27°C. overnight.

(4) Jackson and Gillis method No. IV, in which sodium chloride is added to the solution for direct polarization, and the invert polarization is made in acid solution. The basic factor for this method, according to Jackson and Gillis, is 142.63. The inversion was carried out at 26°–27°C. overnight.

Jackson and Gillis' method No. III is, according to these authors, not applicable in the presence of invert sugar, and was therefore omitted.

Each of the four methods mentioned was used at three different concentrations—normal weight, half-normal weight, and quarter-normal weight of solids in 100 cc.

As it was not the object of this part of the work to establish inversion factors, but to test the correctness of those found previously and to determine the applicability of the various proposed modifications of the Clerget method to products of diverse nature, it was decided to use such equipment as is ordinarily considered necessary in careful control work rather than any special apparatus permitting the greatest refinements in analysis. For this reason those assisting in this work did not resort to the weighing of the solutions, or to the use of elaborate thermostat equipment, etc. Only the control possible by water-jacketed tubes in a constant temperature laboratory kept at 20°C. was therefore employed. In this way complications arising from temperature corrections were avoided.

All the results given in the tables are the averages of two separate analyses, and, in the case of one of the two laboratories, those of two different analysts.

Although it was found impossible in this year's work to include the sirup containing the non-sucrose constituents of blackstrap, the results presented show that there was an important difference between the invert sugar sirups, the sirup used in one of the laboratories containing such constituents that it really took the place of that which the associate referee had intended to prepare from blackstrap.

While in the calculations of the results no corrections had to be applied for temperature, it was necessary, of course, to correct for differences in concentration. This was done by adding to the respective

basic factor the expression  $0.0676 (m - 13)$ . The concentration coefficient 0.0676 is that generally accepted, and it has been confirmed by Jackson and Gillis. " $m$ " stands for concentration in grams per 100 cc. This concentration may be expressed either as total sugar concentration, or as sucrose concentration only. The calculations given in this report were made both ways (except for sucrose alone) in order to determine which is correct.

## RESULTS ON SUCROSE ALONE.

The final results on sucrose alone (Domino tablets) are given in Table 1.

TABLE 1.  
*Sucrose (Domino tablets).*

INVERTASE METHOD.			
Constant: 132.00 plus 0.0676 ( $m - 13$ ).			
	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
26 grams/100 cc.			
R. T. Balch.....	99 84	-33 17	100 10
C. A. Gamble, G. H. Hardin.....	99 89	-33 10	100.08
			100 09
13 grams/100 cc.			
R. T. Balch.....	49 88	-16 10	49.98
C. A. Gamble, G. H. Hardin.....	49 95	-16 03	49 98
			49.98
6.5 grams/100 cc.			
R. T. Balch.....	24 92	-7 95	24 98
C. A. Gamble, G. H. Hardin.....	24 95	-7 95	25 01
			24.995
ACID METHOD.			
Constant: 133.2 plus 0.0676 ( $m - 13$ ).			
26 grams/100 cc.			
R. T. Balch.....	99 84	-34 16	99 94
C. A. Gamble, G. H. Hardin.....	99 89	-34 21	100 02
			99 98
13 grams/100 cc.			
R. T. Balch.....	49 88	-16 64	49 94
C. A. Gamble, G. H. Hardin.....	49 95	-16 59	49 96
			49.95
JACKSON AND GILLIS METHOD No. II.			
Constant: 133.34 plus 0.0676 ( $m - 13$ ).			
26 grams/100 cc.			
R. T. Balch.....	99 30	-34 76	99 88
C. A. Gamble, G. H. Hardin.....	99 39	-34 77	99.96
			99 92
13 grams/100 cc.			
R. T. Balch.....	49 60	-16.90	49 87
C. A. Gamble, G. H. Hardin.....	49.70	-16.90	49.95
			49.91
6.5 grams/100 cc.			
R. T. Balch.....	24.78	-8.34	24.92
C. A. Gamble, G. H. Hardin.....	24 83	-8.34	24 96
			24 94

## JACKSON AND GILLIS METHOD No. IV.

Constant: 132.63 plus 0.0676 ( $m - 18$ ).

<i>26 grams/100 cc.</i>			
R. T. Balch.....	99.35	-34.18	100.02
C. A. Gamble, G. H. Hardin.....	99.34	-34.21	100.03
			<hr/> 100.025
<i>13 grams/100 cc.</i>			
R. T. Balch.....	49.61	-16.64	49.95
C. A. Gamble, G. H. Hardin.....	49.68	-16.59	49.97
			<hr/> 49.96
<i>6.5 grams/100 cc.</i>			
R. T. Balch.....	24.79	-8.22	24.97
C. A. Gamble, G. H. Hardin.....	24.84	-8.15	24.96
			<hr/> 24.965

All four methods tested yield, in general, satisfactory results. It is noted, however, that Jackson and Gillis method No. II tends to give lower values than the other three. In a number of determinations made in this laboratory, it has been found that it is very difficult to obtain good checks on the invert readings with this method. A great deal seems to depend on the exact way in which the neutralization with ammonia is carried out. At times the results agreed exactly with those of Jackson and Gillis, while more often they were lower. Evidently the greatest care is necessary, even with ammonia, to avoid a local destructive effect on the invert sugar.

It is observed, likewise, that the Clerget values for the normal weight per 100 cc. average higher than those for the half-normal weight, while those for the quarter-normal weight average lower. This would seem to indicate that the concentration coefficient 0.0676 is slightly low. It must, of course, be kept in mind that this coefficient represents only an average figure, which in reality varies with the concentration, since the equation for the specific rotation of invert sugar denotes a parabolic curve. A calculation based on Vosburgh's measurements of the specific rotation of invert sugar indicates a coefficient of 0.098 between 6.5 and 13 grams of inverted sucrose per 100 cc., and of 0.088 between 13 and 19 grams per 100 cc. This subject evidently requires further careful study. It is quite possible, and even probable, that the concentration factor is not the same for different modifications of the Clerget method, as indicated by Sazavsky's results.

## RESULTS ON INVERT SIRUP A ALONE.

Since it was found necessary to prepare two different invert sirups, which showed on analysis a different composition, the two will be taken up separately. Sirup A showed the percentages of sucrose presented in Table 2.

TABLE 2.

*Invert sugar sirup A (R. T. Balch).*

Total solids = 62.85° Brix.

("C" = grams solids/100 cc.; "S" = grams sucrose/100 cc.)

INVERTASE METHOD.				
Constant: 132.0 plus 0.0676 ( $m - 13$ ).				
	DIRECT POLARIZATION	INVERT POLARIZATION	SUCROSE IN SIRUP, $m = C$ per cent	SUCROSE IN SIRUP, $m = S$ per cent
26 grams sirup/100 cc.....	-19 45	-19.50	0 04	0 04
13 grams sirup/100 cc.....	- 9 53	- 9.55	0.03	0 03
6.5 grams sirup/100 cc.....	- 4.76	- 4.77	0.03	0.03
			0.03	0 03
ACID METHOD.				
Constant: 133.2 plus 0.0676 ( $m - 13$ ).				
26 grams sirup/100 cc.....	-19 45	-20 22	0.58	0 59
13 grams sirup/100 cc.....	- 9 53	- 9.91	0 58	0 58
6.5 grams sirup/100 cc.....	- 4.76	- 4.94	0 55	0 55
			0 57	0 57
JACKSON AND GILLIS METHOD No. II.				
Constant. 133.34 plus 0.0676 ( $m - 13$ ).				
26 grams sirup/100 cc.....	-20 66	-20 70	0 03	0 03
13 grams sirup/100 cc.....	-10 05	-10.10	0 08	0 08
6.5 grams sirup/100 cc.....	- 5 02	- 5 02	0 00	0 00
			0 04	0 04
JACKSON AND GILLIS METHOD No. IV.				
Constant: 132.63 plus 0.0676 ( $m - 13$ ).				
26 grams sirup/100 cc.....	-20 15	-20 22	0 05	0 05
13 grams sirup/100 cc.....	- 9 88	- 9 91	0 05	0 05
6.5 grams sirup/100 cc.....	- 4 94	- 4 94	0 00	0 00
			0 03	0 03

This sirup was evidently practically free from sucrose. If it be accepted as a fact that the Clerget method, in which invertase is used, gives the true sucrose content, then both of the Jackson and Gillis methods used also give correct results with a sirup of this nature. The slight deviations shown are well within the limits of error, no matter what concentration was employed. Moreover, the percentage of sucrose is so small that the same results are obtained whether the calculations are based on sucrose concentration alone, which is practically zero, or on total sugar concentration, which varied from about 4-16 grams of solids per 100 cc., with corresponding differences in the divisors.

As is already well known, the plain acid method gives too high results, on account of the effect of the free acid on the rotation of the levulose. For this reason an apparent sucrose content of 0.57 per cent is found against an average of only 0.03 per cent by the other three methods.

Whether the normal, half-normal, or quarter-normal weight is used for analysis, the results are, within the limits of error, the same.

It may be concluded, therefore, that small quantities of sucrose in mixture with invert sugar only, can be determined by the Jackson and Gillis methods used in this work, as well as by the invertase method, but that the plain acid method gives results very much too high.

#### RESULTS ON MIXTURES OF SUCROSE AND INVERT SIRUP A.

The results for four such mixtures are given in Table 3. The percentages of sucrose for each mixture, calculated on the basis of the figures recorded in Tables 1 and 2, are always given first, followed by the results of the actual analyses. It will be noted that the calculated values for the invertase methods, Jackson and Gillis method No. II, and Jackson and Gillis method No. IV, are practically identical, while those calculated for the plain acid method are naturally too high and therefore fictitious. The found values are given, as before, on the basis of sucrose concentration alone, and on that of total sugar concentration.

TABLE 3.  
*Mixtures of sucrose and invert sugar sirup A (R. T. Balch).*

MIXTURE I.				
Three parts sucrose and one part invert sirup solids.				
Sucrose in mixture, calculated from sucrose found in components:				
			<i>per cent</i>	
By invertase method.....			74.94	
By acid method.....			75.15	
By Jackson and Gillis method No. II.....			74.94	
By Jackson and Gillis method No. IV.....			74.94	
	DIRECT POLARIZATION	INVERT POLARIZATION	SUCROSE <i>m</i> = <i>C</i>	SUCROSE <i>m</i> = <i>S</i>
<i>Invertase method</i>			<i>per cent</i>	<i>per cent</i>
26 grams solids/100 cc.....	66.84	-32.85	75.01	75.27
13 grams solids/100 cc.....	33.60	-15.94	74.91	75.03
6.5 grams solids/100 cc.....	16.84	-7.83	75.01	75.07
			74.98	75.12
<i>Acid method</i>				
26 grams solids/100 cc.....	66.84	-33.86	75.11	75.35
13 grams solids/100 cc.....	33.60	-16.42	75.11	75.23
6.5 grams solids/100 cc.....	16.84	-8.10	75.14	75.19
			75.12	75.26
<i>J. and G. method No. II</i>				
26 grams solids/100 cc.....	66.09	-34.40	74.87	75.12
13 grams solids/100 cc.....	33.12	-16.78	74.85	74.97
6.5 grams solids/100 cc.....	16.64	-8.22	74.82	74.99
			74.85	74.99
<i>J. and G. method No. IV</i>				
26 grams solids/100 cc.....	66.17	-33.86	74.92	75.17
13 grams solids/100 cc.....	33.20	-16.42	74.83	74.95
6.5 grams solids/100 cc.....	16.68	-8.10	74.98	75.05
			74.91	75.06

## MIXTURE II.

One part sucrose and one part invert sirup solids.

Sucrose in mixture, calculated from sucrose found in components:

			<i>per cent</i>	
By invertase method.....			49.98	
By acid method.....			50.42	
By Jackson and Gillis method No. II.....			49.98	
By Jackson and Gillis method No. IV.....			49.98	
<i>Invertase method</i>				
26 grams solids/100 cc.....	34 00	-32 48	50.03	50 36
13 grams solids/100 cc.....	17 24	-15 63	49 80	49 97
6.5 grams solids/100 cc.....	8 70	- 7 74	49 98	50.07
			49 97	50 13
<i>Acid method</i>				
26 grams solids/100 cc.....	34 00	-33 65	50.45	50.79
13 grams solids/100 cc.....	17 24	-16 30	50.36	50 53
6.5 grams solids/100 cc.....	8 70	- 8.05	50 47	50 55
			50 43	50 62
<i>J. and G. method No. II</i>				
26 grams solids/100 cc.....	32 88	-34 09	49 90	50.23
13 grams solids/100 cc.....	16 69	-16 61	49 95	50.11
6.5 grams solids/100 cc. ....	8 43	- 8 22	50 11	50 20
			49 99	50 18
<i>J. and G. method No. IV</i>				
26 grams solids/100 cc. ....	33 13	-33 65	50 02	50 35
13 grams solids/100 cc.....	16 79	-16 30	49 90	50.06
6.5 grams solids/100 cc.....	8 47	- 8 05	49 99	50 07
			49 97	50 16

## MIXTURE III.

One part sucrose and three parts invert sirup solids.

Sucrose in mixture, calculated from sucrose found in components:

			<i>per cent</i>	
By invertase method.....			25.03	
By acid method.....			25.69	
By Jackson and Gillis method No. II.....			25.04	
By Jackson and Gillis method No. IV.....			25.03	
<i>Invertase method</i>				
26 grams solids/100 cc.....	1 13	-32 11	25 02	25 27
13 grams solids/100 cc.....	0 99	-15 55	25 06	25 19
6.5 grams solids/100 cc.....	0 60	- 7 65	25 08	25 15
			25 05	25 20
<i>Acid method</i>				
26 grams solids/100 cc.....	1 13	-33 22	25 62	25 87
13 grams solids/100 cc.....	0 99	-16 13	25 71	25 83
6.5 grams solids/100 cc.....	0 60	- 7 96	25 79	25 85
			25 71	25 85
<i>J. and G. method No. II</i>				
26 grams solids/100 cc.....	0 34	-33 79	24 92	25 17
13 grams solids/100 cc.....	0 29	-16 45	25.09	25.21
6.5 grams solids/100 cc.....	0 21	- 8 10	25.01	25.07
			25.01	25 15
<i>J. and G. method No. IV</i>				
26 grams solids/100 cc.....	0 15	-33 22	24 99	25 24
13 grams solids/100 cc.....	0 47	-16 13	25 03	25 16
6.5 grams solids/100 cc.....	0 33	- 7 96	25 08	25 15
			25.03	25 18

## MIXTURE IV.

13 parts sucrose and 8.17 parts invert sirup solids.

Sucrose in mixture, calculated from analysis of components:

			<i>per cent</i>	
	By invertase method.....		61.36	
	By acid method.....		61.70	
	By Jackson and Gillis method No. II.....		61.37	
	By Jackson and Gillis method No. IV.....		61.36	
<i>Invertase method</i>				
	21.17 grams solids/100 cc.....	40.07	-26.28	61.48
	10.59 grams solids/100 cc.....	20.21	-12.74	61.44
	5.29 grams solids/100 cc.....	10.10	- 6.30	61.28
				61.40
				61.54
<i>Acid method</i>				
	21.17 grams solids/100 cc.....	40.07	-27.28	61.85
	10.59 grams solids/100 cc.....	20.21	-13.23	61.79
	5.29 grams solids/100 cc.....	6.55	- 6.55	61.65
				61.76
				61.89
<i>J. and G. method No. II</i>				
	21.17 grams solids/100 cc.....	39.23	-27.74	61.43
	10.59 grams solids/100 cc.....	19.75	-13.51	61.39
	5.29 grams solids/100 cc.....	9.92	- 6.69	61.44
				61.42
				61.56
<i>J. and G. method No. IV</i>				
	21.17 grams solids/100 cc.....	39.40	-27.28	61.49
	10.59 grams solids/100 cc.....	19.89	-13.23	61.46
	5.29 grams solids/100 cc.....	9.98	- 6.55	61.47
				61.47
				61.61

With any of the four mixtures and by any of the four methods employed, the average results for the three concentrations used give, within the limits of error, satisfactory agreement with those calculated, always on the basis of the same method, provided, however, that in these calculations the divisor corresponding to the total sugar concentration, and not that for the sucrose concentration, is used. The latter way of calculating gives high results, the greatest discrepancies being observed when the sucrose and invert sirup solids are about equal in quantity, or when the total sugar concentration per 100 cc. is high.

With any of the four modifications of the Clerget method used, it does not seem to make any difference whether the solution contains the full-, half-, or quarter-normal weight. While it is true that in individual cases the average results of duplicate analyses differ sometimes by more than 0.1 from the calculated, nevertheless there is noticeable no systematic deviation.

The conclusions to be drawn for the mixtures of sucrose with this invert sugar sirup A are therefore the same as those stated for the invert sugar itself, viz., that the invertase method and the Jackson and Gillis methods No. II and IV give correct results within the limits of error, provided, however, that the calculations are based on total sugar concentration, and not on sucrose concentration alone.

## RESULTS ON INVERT SUGAR SIRUP B.

As this sirup gave anomalous results when analyzed, its preparation will be described more fully. The method given in Scientific Paper 375<sup>1</sup> was followed in general—a sucrose solution of about 50 Brix being inverted at 50° with invertase. The particular sample of invertase of high activity used was furnished by the Wallerstein Laboratories, and the writer wishes to express his thanks to this firm for their courtesy. When no further change in the polarization of the sirup could be detected within a 24 hour period, the inversion was considered complete. The sirup was then heated for an hour to 70°–80°C. to destroy the invertase. As the color had darkened perceptibly, 2 per cent of “Suchar” on total solids was added, and the sirup was filtered and finally brought to pH 7 by careful neutralization with sodium carbonate. The concentration was found to be equivalent to 70 Brix. As the sirup had been kept in the refrigerator for about two weeks and had begun to show signs of crystallization, it was diluted to 52.05 Brix by addition of distilled water. At this concentration no further change occurred.

The analyses of this sirup are shown in Table 4.

The composition of this sirup was evidently quite different from that of Sirup A. In the first place, it still contained 0.29 per cent of uninverted sucrose, as shown by inversion with invertase. As was expected, the plain acid method gave higher results than the invertase method, but the difference was much greater than had been found in Sirup A. This difference indicates that there was present, besides sucrose, some substance that was hydrolyzed by the acid and not by the invertase. This conclusion is further strengthened by the fact that both of the Jackson and Gillis methods gave higher results than the invertase method. Taking into consideration the difference in the concentration of the two sirups, the plain acid method should have shown 0.43 per cent more apparent sucrose than the invertase method, or altogether 0.72 per cent. It actually gave 1.18 per cent, or 0.46 per cent more than that. This apparent excess sucrose was again found by the two Jackson and Gillis methods, these giving an average of 0.44 per cent more than the invertase method. It is reasonable to conclude that this apparent excess sucrose is really simulated by reversion products formed in the invert sirup by heating first for days to 50°C. and finally for an hour to a maximum of 80°C.

The results by Jackson and Gillis method No. II are slightly lower than those by No. IV, probably for the reason already discussed in connection with the analysis of sucrose.

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<sup>1</sup> U. S. Bur. Standards Sci. Paper 375.



TABLE 4.

*Invert sugar sirup B (C. A. Gamble, G. H. Hardin).*

INVERTASE METHOD.				
Constant: 132.0 plus 0.0676 ( $m - 13$ ).				
	DIRECT POLARIZATION	INVERT POLARIZATION	SUCROSE, $m = C$ per cent	SUCROSE, $m = S$ per cent
27.07 grams solids/100 cc.....	-31.37	-32.14	0.29	0.29
18.20 grams solids/100 cc.....	-20.73	-21.22	0.27	0.27
13.53 grams solids/100 cc.....	-15.13	-15.52	0.30	0.30
6.77 grams solids/100 cc.....	- 7.41	- 7.62	0.32	0.32
3.38 grams solids/100 cc.....	- 3.68	- 3.77	0.27	0.27
			0.29	0.29
ACID METHOD.				
Constant: 133.2 plus 0.0676 ( $m - 13$ ).				
27.07 grams solids/100 cc.....	-31.37	-34.52	1.17	1.19
18.20 grams solids/100 cc.....	-20.73	-22.82	1.16	1.17
13.53 grams solids/100 cc.....	-15.13	-16.70	1.18	1.18
6.77 grams solids/100 cc.....	- 7.41	- 8.14	1.10	1.10
3.38 grams solids/100 cc.....	- 3.68	- 4.10	1.27	1.27
			1.18	1.18
JACKSON AND GILLIS METHOD No. II.				
Constant: 133.34 plus 0.0676 ( $m - 13$ ).				
27.07 grams solids/100 cc.....	-33.32	-35.02	0.63	0.64
18.20 grams solids/100 cc.....	-21.93	-23.11	0.66	0.67
13.53 grams solids/100 cc.....	-16.07	-16.97	0.68	0.68
6.77 grams solids/100 cc.....	- 7.86	- 8.36	0.75	0.75
3.38 grams solids/100 cc.....	- 3.90	- 4.16	0.78	0.78
			0.70	0.70
JACKSON AND GILLIS METHOD No. IV.				
Constant: 132.63 plus 0.0676 ( $m - 13$ ).				
27.07 grams solids/100 cc.....	-32.51	-34.52	0.75	0.76
18.20 grams solids/100 cc.....	-21.49	-22.82	0.74	0.75
13.53 grams solids/100 cc.....	-15.72	-16.70	0.74	0.74
6.77 grams solids/100 cc.....	- 7.65	- 8.14	0.74	0.74
3.38 grams solids/100 cc.....	- 3.84	- 4.10	0.79	0.79
			0.75	0.76

Determinations made at different concentrations agree satisfactorily with each other, except for the lowest concentrations, where the errors of observation are greatly multiplied.

If, as is reasonable, the invertase method is taken as a standard, the conclusion may fairly be drawn that in the presence of reversion products any method in which acid is used for inversion, at temperatures only slightly above ordinary room temperatures, these reversion products will be hydrolyzed and make it appear that sucrose is present when none is really there. This conclusion has also been arrived at independently by Bruhns<sup>1</sup>. When higher temperatures are used for inversion, the condi-

<sup>1</sup> Chem. Ztg., 1921, 45: 661, 681, 685, 711.

tions may become very complicated; here hydrolysis and condensation may go on simultaneously, and it is quite possible that through a compensation of two errors the methods of acid hydrolysis with, or even without, neutralization of the acid, may give correct results. This question requires further study.

#### RESULTS ON MIXTURES OF SUCROSE AND INVERT SIRUP B.

These mixtures were prepared to correspond exactly to Mixtures I, II, and III, in which Sirup A was used. The analyses of the mixtures with Sirup B are shown in Table 5.

TABLE 5.

*Mixtures of sucrose and invert sugar sirup B (C. A. Gamble, G. H. Hardin).*

MIXTURE I.					
Three parts sucrose and one part invert sirup solids.					
Sucrose in mixture, calculated from sucrose found in components:					
			<i>per cent</i>		
By invertase method. . . . .			75.08		
By acid method. . . . .			75.46		
By Jackson and Gillis method No. II. . . . .			75.23		
By Jackson and Gillis method No. IV. . . . .			75.27		
	DIRECT POLARIZATION	INVERT POLARIZATION	SUCROSE, $m = C$	SUCROSE, $m = S$	
<i>Invertase method</i>					
26 grams solids/100 cc. . . . .	67 21	-32 68	75 17	75 42	
13 grams solids/100 cc. . . . .	33 74	-15 86	75 15	75 28	
6.5 grams solids/100 cc. . . . .	16 91	- 8 16	(76 22)*	(76 29) *	
			75 16	75 35	
<i>Acid method</i>					
26 grams solids/100 cc. . . . .	67.21	-33 94	75 44	75 69	
13 grams solids/100 cc. . . . .	33 74	-16 48	75 40	75 53	
6.5 grams solids/100 cc. . . . .	16 91	- 8 12	75 41	75 48	
			75 42	75.57	
<i>J. and G. method No. II</i>					
26 grams solids/100 cc. . . . .	66.34	-34 57	75 18	75 43	
13 grams solids/100 cc. . . . .	33.28	-16 82	75 15	75 27	
6.5 grams solids/100 cc. . . . .	16.75	- 8.24	75 22	75.28	
			75.18	75.33	
<i>J. and G. method No. IV</i>					
26 grams solids/100 cc. . . . .	66 50	-33 94	75.23	75 48	
13 grams solids/100 cc. . . . .	33.37	-16 48	75 17	75.30	
6.5 grams solids/100 cc. . . . .	16.75	- 8 12	75.26	75.32	
			75.22	75.37	

## MIXTURE II.

One part sucrose and one part invert sirup solids.

Sucrose in mixture, calculated from sucrose found in components:

			<i>per cent</i>	
By invertase method.....			50.25	
By acid method.....			51.08	
By Jackson and Gillis method No. II.....			50.62	
By Jackson and Gillis method No. IV.....			50.65	
<i>Invertase method</i>				
26 grams solids/100 cc.....	34.73	-31.95	50.17	50.52
13 grams solids/100 cc.....	17.69	-15.47	50.24	50.40
6.5 grams solids/100 cc.....	8.85	-7.79	(50.59)*	(50.68)*
			50.21	50.46
<i>Acid method</i>				
26 grams solids/100 cc.....	34.73	-33.66	50.99	51.34
13 grams solids/100 cc.....	17.69	-16.25	50.96	51.13
6.5 grams solids/100 cc.....	8.85	-8.03	50.86	50.94
			50.94	51.14
<i>J. and G. method No. II</i>				
26 grams solids/100 cc.....	33.52	-34.24	50.48	50.82
13 grams solids/100 cc.....	17.02	-16.69	50.56	50.73
6.5 grams solids/100 cc.....	8.62	-8.20	50.63	50.71
			50.56	50.75
<i>J. and G. method No. IV</i>				
26 grams solids/100 cc.....	33.96	-33.66	50.65	50.98
13 grams solids/100 cc.....	17.26	-16.25	50.53	50.70
6.5 grams solids/100 cc.....	8.70	-8.03	50.62	50.71
			50.60	50.80

\* Not included in average.

## MIXTURE III.

One part sucrose and three parts invert sirup solids.

Sucrose in mixture, calculated from sucrose found in components:

			<i>per cent</i>	
By invertase method.....			25.39	
By acid method.....			26.62	
By Jackson and Gillis method No. II.....			25.92	
By Jackson and Gillis method No. IV.....			25.04	
<i>Invertase method</i>				
26 grams solids/100 cc.....	2.37	-31.25	25.30	25.55
13 grams solids/100 cc.....	1.53	-15.18	25.32	25.45
6.5 grams solids/100 cc.....	0.85	-7.49	25.36	25.42
			25.33	25.47
<i>Acid method</i>				
26 grams solids/100 cc.....	2.37	-33.22	26.54	26.80
13 grams solids/100 cc.....	1.53	-16.16	26.56	26.69
6.5 grams solids/100 cc.....	0.85	-7.97	26.57	26.64
			26.56	26.71
<i>J. and G. method No. II</i>				
26 grams solids/100 cc.....	0.90	-33.75	25.82	26.05
13 grams solids/100 cc.....	0.77	-16.49	25.89	26.02
6.5 grams solids/100 cc.....	0.51	-8.08	25.85	25.92
			25.85	26.00
<i>J. and G. method No. IV</i>				
26 grams solids/100 cc.....	1.37	-33.22	25.91	26.17
13 grams solids/100 cc.....	1.00	-16.16	25.88	26.01
6.5 grams solids/100 cc.....	0.60	-7.97	25.93	26.00
			25.91	26.06

The average results found by each method again agree satisfactorily with those calculated on the basis of the *same* method, provided that the divisor corresponding to total sugar concentration and not to sucrose concentration is used.

In order to give a better idea of these relationships, Table 6 has been compiled, in which are presented the average differences between found and calculated values for the various mixed products, with both Sirup A and Sirup B. These differences are given on the basis of sucrose concentration as well as of total sugar concentration. It is noted that when the latter basis is used, the found values average 0.01 per cent below the calculated, while on the basis of sucrose alone the general average by all methods is 0.16 per cent too high.

TABLE 6.

*Average differences between sucrose in mixtures, calculated, and average values found.*

	INVERTASE METHOD	ACID METHOD	J. AND G. METHOD NO. II	J. AND G. METHOD NO. IV	
Divisor based on "C"					
Mixture I . . . . .	+0 06	-0 04	-0 07	-0 04	
Mixture II . . . . .	-0 03	-0 08	-0 03	-0 03	
Mixture III . . . . .	-0 02	-0 02	-0 05	-0 07	
Mixture IV . . . . .	+0 04	+0 06	+0 05	+0 11	
Averages . . . . .	+0 01	-0 02	-0 03	-0 01	
General average . . . . .					-0 01
Divisor based on "S"					
Mixture I . . . . .	+0 23	+0 11	+0 08	+0 11	
Mixture II . . . . .	+0 18	+0 13	+0 17	+0 17	
Mixture III . . . . .	+0 13	+0 13	+0 15	+0 09	
Mixture IV . . . . .	+0 18	+0 19	+0 19	+0 25	
Averages . . . . .	+0 18	+0 14	+0 16	+0 16	
General average . . . . .					+0 16

Table 5 also shows that in each of the four methods, the normal, half-normal, and quarter-normal weight give, within the limits of error, the same results.

But when the results of the four methods are compared, it is found, as with invert sugar Sirup B, that the invertase method gives the lowest figures. Taking these as the standard, the figures obtained by the two Jackson and Gillis methods are too high, and those by the plain acid method very much too high. The extent of the error naturally depends on the relative quantity of sirup mixed with the sucrose. In mixture No. I the proportion is so small that the results by the two Jackson and Gillis methods, if calculated on the proper basis, are, within the limits of error, identical with those of the invertase method.

It has not been possible to carry out any work on clarification of saccharine products previous to polarimetric analysis. However, as Jackson and Gillis have pointed out, if it is considered necessary to delead the solution used for the invert reading, the same should be done to the solution used for the direct reading, in order to have conditions as nearly alike as possible. The recommendations hereafter given are formulated accordingly.

#### RECOMMENDATIONS<sup>1</sup>.

On the basis of the work of previous investigators and the further confirmation of their results in this year's collaborative study of polariscopic methods, the associate referee respectfully offers the following recommendations:

(1) That the text in the *Book of Methods*, giving directions for the determination of sucrose by inversion with hydrochloric acid, in the absence of raffinose, be amended to read as follows:

##### DETERMINATION OF SUCROSE IN THE ABSENCE OF RAFFINOSE.

(In the presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup and molasses, the optical method for sucrose, using hydrolysis by acid (21), gives high results.)

#### 21 *By Polarization Before and After Inversion With Hydrochloric Acid.—Official.*

Dissolve the double normal weight (52 grams) of the substance in water in a 200 cc. flask; add basic lead acetate carefully, avoiding any excess, then 1–2 cc. alumina cream; shake; dilute to the mark with water; mix well; and filter, rejecting at least the first 25 cc. of the filtrate. Cover the funnel with a watch glass. When sufficient filtrate has collected, remove the lead from the solution by adding dry, powdered potassium oxalate, a little at a time, avoiding any excess; mix well; and filter again, rejecting at least the first 25 cc. of the filtrate. (Instead of weighing 52 grams into a 200 cc. flask, two 26 gram portions may be made up to 100 cc. each, and treated exactly as described. Depending on the color of the product, multiples or fractions of the normal weight may be used, and the results reduced by calculation to the basis of 26 grams in 100 cc.)

Pipet one 50 cc. portion of the lead-free filtrate into a 100 cc. flask, dilute with water to the mark, mix well, and polarize in a 200 mm. tube. The result, multiplied by 2, is the direct reading ( $P$  of formula given below) or polarization before inversion. (If a 400 mm. tube is used, the reading equals  $P$ .) See — concerning mutarotation.

For the invert reading, pipet another 50 cc. portion of the lead-free filtrate into a 100 cc. flask, and add 25 cc. of water. Then add, little by little, while rotating the flask, 5 cc. of hydrochloric acid of sp. gr. 1.1918 at 20°/4°C., or 10 cc. of the acid of sp. gr. 1.1029 at 20°/4°C. Heat a water bath to 70°C. and regulate the burner so that the temperature of the bath remains approximately at that point. Place the flask in the water bath, insert a thermometer, and heat with constant agitation until the thermometer in the flask indicates 67°C. This preliminary heating period should require from 2½–2¼ minutes. From the moment the thermometer in the flask indicates 67°C., leave the flask in the bath for exactly 5 minutes longer, during which time the temperature should gradually rise to about 69.5°C. Plunge the flask at once into water at 20°C. When the contents have cooled to about 35°C., remove the thermometer from the flask,

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 259.

rinse it, and fill almost to the mark. Leave the flask in the bath at 20°C. for at least 30 minutes longer, and finally make up exactly to volume. Mix well and polarize the solution in a 200 mm. tube provided with a lateral branch and a water jacket, maintaining a temperature of 20°C. This reading must also be multiplied by 2 to obtain the invert reading. If it is necessary to work at a temperature other than 20°C., which is permissible within narrow limits, the volumes must be completed and both direct and invert polarizations must be made at exactly the same temperature.

Calculate sucrose by the following formula:

$$S = \frac{100 (P - I)}{143 + 0.0676 (m - 13) - T/2}, \text{ in which}$$

$S$  = percentage of sucrose;

$P$  = direct reading, normal solution;

$I$  = invert reading, normal solution;

$T$  = temperature at which readings are made; and

$m$  = grams of total solids in 100 cc. of the invert solution read in the polariscope.

Determine total solids as percentage by weight, as directed under 7, and multiply by the specific gravity as obtained from Table —, page —.

The inversion may also be accomplished as follows:

(1) To 50 cc. of the clarified solution, freed from lead, add 5 cc. of hydrochloric acid of sp. gr. 1.1918, or 10 cc. of the acid of sp. gr. 1.1029 and set aside for 24 hours at a temperature not below 20°C.; or, (2) if the temperature is above 25°C., set aside for 10 hours. Make up to 100 cc. at 20°C. and polarize as directed above. Under these conditions the formula must be changed to the following:

$$S = \frac{100 (P - I)}{143.2 + 0.0676 (m - 13) - T/2}.$$

(2) That the text in the *Book of Methods*, giving directions for the method of sucrose determination by inversion with invertase, in the absence of raffinose, be amended to read as follows:

## 23

### DETERMINATION.

Prepare solution of product and make direct polarization as described in 21, first and second paragraphs. See — concerning mutarotation.

To another 50 cc. portion of the lead-free filtrate in a 100 cc. volumetric flask add glacial acetic acid, drop by drop, until the reaction is acid to litmus. Add 10 cc. of the invertase solution, fill the flask with water nearly to 100 cc., and let stand in a warm place (about 40°C.) overnight. Cool, and make up to 100 cc. at 20°C. Mix well and polarize at 20°C. in a 200 mm. tube. Allow the solution to remain in the tube for an hour and repeat the polarization. If there is no change from the previous reading, the inversion is complete, whereupon the reading and temperature of the solution are carefully noted. Correct the polarization for the optical activity of the invertase solution and multiply by 2. Calculate the percentage of sucrose by the following formula:

$$S = \frac{100 (P - I)}{142 + 0.0676 (m - 13) - T/2}, \text{ in which}$$

$S$  = percentage of sucrose;

$P$  = direct reading, normal solution;

$I$  = invert reading, normal solution;

$T$  = temperature at which readings are made;

$m$  = grams of total solids in 100 cc. of the invert solution read in the polariscope

Determine the total solids as percentage by weight, as directed under 7, and multiply this figure by the specific gravity at 20°/4°C., as obtained from Table —, page —.

(3) That a further study be made of the analysis of sugar mixtures, and especially of mixtures containing besides sucrose and invert sugar, amino acids and other impurities, as well as raffinose. Such a study should include not only the present methods of the association, but also those recommended by Jackson and Gillis and by others; special attention should be given to inversion at higher temperatures.

## REPORT ON CHEMICAL METHODS FOR REDUCING SUGAR.

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),  
*Associate Referee.*

The reducing sugar methods that have been compiled for this association represent the results of the work of numerous collaborators over a period of many years and show the effects of the careful consideration extended by previous referees. The work of the present associate referee during the past year has consisted of a general survey of the subject for the purpose of suggesting a revision of the reducing sugar methods. As a result of this survey it appears that the suggested activities may be classified under three heads, which will be considered in turn. (1) Incorporation of the results of recent research; (2) discarding duplicate or obsolete methods; (3) an examination of the limits of accuracy of the present methods and a scrutiny and verification of the present standard reference tables.

In recent years important researches have been published on the volumetric estimation of reducing sugar. The discovery has been made by Lane and Eynon<sup>1</sup> that methylene blue when used as an inside indicator is rapidly decolorized by a slight excess of sugar, the reaction constituting a readily discernible end point. This, the first suggested method of convenient inside indication, has been uniformly reported favorably by those who have attempted its application.

Other methods have been described in which a portion of the known total copper has been reduced by sugar, and a volumetric method has been employed to determine either the quantity of excess unreduced copper or the quantity of reduced copper. The determination in either case is made iodimetrically. In some instances the estimation is made, after filtration, either on the precipitated cuprous oxide or on the filtrate after acidification. In other instances the titration is performed after the completion of the reduction, directly in the reaction vessel, without a previous filtration<sup>2</sup>. The great convenience of these methods serves as their own recommendation.

<sup>1</sup> *J. Soc. Chem. Ind.*, 1923, 42: 32.

<sup>2</sup> e. g., W. B. Clark, *J. Am. Chem. Soc.*, 1918, 40: 1759; Scales, *J. Ind. Eng. Chem.*, 1919, 11: 747; Shaffer and Hartmann, *J. Biol. Chem.*, 1921, 45: 349.

Other authors have devoted their efforts to a great elaboration of procedure, such as will yield results of high precision even at a considerable sacrifice of convenience<sup>1</sup>. It is the opinion of the associate referee that the association's list should contain one method of high precision, which may be applicable to the occasion when such precision is required.

A number of well established methods are in existence which are substantially micro-chemical<sup>2</sup>. Inasmuch as these may be applied to studies of plant metabolism and similar subjects and thus be adaptable to the work of this association, it is recommended that one such analytical process be added to the reducing sugar methods.

There has been considerable study in the past few years of a method for the determination of aldose sugars by oxidation in a weakly alkaline medium by iodine<sup>3</sup>. As an instance of its application, it is possible to determine dextrose in the presence of levulose by direct titration. While this procedure is still in the experimental stage, it is suggested that a preliminary study be made with a view to its ultimate inclusion.

Passing on to the second subject, it may be noted that in the present group of methods there is apparently a partial duplication. Meissl's method for invert sugar is an almost exact duplicate of that of Munson and Walker. Wein's method for maltose differs from that of Munson and Walker only in the time of boiling and, similarly, Soxhlet and Wein's procedure for lactose differs from that of Munson and Walker, a six-minute period of boiling being required instead of a two-minute period. Allihn's method for glucose duplicates Munson and Walker's method except for a variation in the composition of the reagents. Among the methods for the determination of copper there are three procedures for the electrolytic separation varying in respect to the acidity of the medium. The referee suggests that so extensive a duplication is not only prodigal of space but is confusing to the analyst. It is recommended that the duplicate methods be intercompared and that of each pair the one more suitable be selected and the remaining one be discarded.

Third. The question is frequently raised as to what degree of precision may be expected in reducing sugar analysis. It is suggested, therefore, that a profitable study would be a determination of the magnitude of the errors in the respective methods, with a view to expressing numerically in the text the limits of accuracy that should be expected of the analyst.

Finally, while no doubt is implied in regard to the accuracy of the standard reference tables, it is suggested that in common with all empirical data they should be subjected to constant scrutiny.

<sup>1</sup> e. g., Quisumbing and Thomas, *J. Am. Chem. Soc.*, 1921, 43: 1503.

<sup>2</sup> Folin and Wu, *J. Biol. Chem.*, 1919, 38: 81; 1920, 41: 367

<sup>3</sup> e. g., Willstätter and Schudel, *Ber.*, 1918, 51: 780; Bougault, *J. Pharm. et Chim.*, 1917, Series 7, 16: 97; Cajorie, *J. Biol. Chem.*, 1922, 54: 617.



To sum up a tentative program, which obviously will require many years to complete, it is recommended that the following subjects be studied:

- (1) The volumetric method in which methylene blue is employed as an inside indicator.
- (2) The volumetric method in which the reduced copper or residual unreduced copper is determined iodimetrically.
- (3) The method of Quisumbing and Thomas or an equivalent method of high precision.
- (4) A micro-chemical method.
- (5) The iodine method for aldose sugars.
- (6) The electrolytic method for copper.
- (7) The reduction of copper in alcohol vapor.
- (8) A selective comparison of the methods now given in practical duplication.
- (9) The limits of accuracy of the respective methods.
- (10) A verification of the standard reference tables.

#### COMMITTEES NAMED BY THE PRESIDENT.

*Committee on nominations:* A. J. Patten of Michigan, G. W. Hoover of Washington, D. C., and W. F. Hand of Mississippi.

*Committee on resolutions:* G. S. Fraps of Texas, W. M. Allen of North Carolina, and A. P. Kerr of Louisiana.

*Auditing committee:* H. H. Hanson of Delaware, and J. H. Mitchell of South Carolina.

*Committee to wait upon Secretary of Agriculture:* C. H. Jones of Vermont, and H. B. McDonnell of Maryland.

*Committee to wait upon the Honorary President:* C. A. Browne of Washington, D. C., and B. B. Ross of Alabama.

FIRST DAY.  
MONDAY—AFTERNOON SESSION.

REPORT ON FERTILIZERS<sup>1</sup>.

By R. N. BRACKETT (Clemson Agricultural College, Clemson College,  
S. C.), *General Referee*.

The report of Sub-committee A on Recommendations of Referees<sup>2</sup> shows that the Ross-Deemer method for water-soluble boric acid, the Bartlett method for total boric acid, and the Robinson procedure for the preparation of ammonium citrate were approved and adopted as official (final action). It was suggested that the Devarda and Moore methods for the determination of nitrogen be further studied. No report and recommendations were made on potash by any referee. It may be recalled, however, that the general referee, by order of the association, made a report on the Lindo-Gladding method, with the recommendation that no change be made in the method of washing the potash precipitate.

As there were no recommendations on potash, the associate referee, A. P. Kerr, did an interesting piece of work on a modification of the Lindo-Gladding method. He will present a report of this work later.

R. B. Deemer was unable to serve as referee and W. H. Ross of the Bureau of Soils, Washington, D. C., was appointed. The work assigned to Ross was suggested by a paper by E. L. Larison<sup>3</sup>, who was urging a change in Paragraph 6 of the chapter on Fertilizers—that of neutralizing with hydrochloric acid before precipitating with the magnesium mixture. On the recommendation of the referee last year, this change was approved under suspension of the rules. After the adjournment of the meeting, valid objections were raised on several sides to the incorporation of this change in the revised *Book of Methods*. The general referee, therefore, requested R. E. Doolittle, chairman of the Committee on Editing Methods of Analysis, not to incorporate this change. Work along this line was undertaken by Ross, who will make a report. J. M. McCandless, of Atlanta, Ga., also did some work on this subject which was referred to Ross.

The general referee wishes also to say that the change suggested in Paragraph 10 of the chapter on Fertilizers, Gravimetric Method for Water-soluble Phosphoric Acid, *viz.*, to allow trituration of the sample, if necessary, with a small quantity of water before washing on a filter, was approved under suspension of the rules. After adjournment of the association several valid objections were raised to the incorporation of this change in the revised *Book of Methods*, and the referee requested

<sup>1</sup> Presented by J. H. Mitchell.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 265

<sup>3</sup> *Ibid.*, 394.

Doolittle not to include it. In this connection it is interesting to note that C. Clifton Howes, of the Davison Chemical Company, who asked for this change, wrote last March, in reply to a request for some samples of the double superphosphate, that a slight modification in their method of manufacture of the double superphosphate made any such change unnecessary, and that the official method was entirely satisfactory.

Referring further to recommendations that concern the revision of methods in the chapter on Fertilizers, which recommendations were offered at the last annual convention, the general referee begs to call the attention of the association to the fact that some of these recommendations were approved for first action of a change in an official method<sup>1</sup>, and they naturally come up for a second action at this meeting. They were in Paragraphs 5, 8, 13, and 48. It is the opinion of the general referee that the changes suggested in the preparation of solution, Paragraph 5, are highly desirable.

In connection with work on potash C. M. Bible, Chief Chemist of the Read Phosphate Company, Nashville, Tenn., sent to the general referee a paper, entitled "A Modification of the Official Lindo-Gladding Method for the Determination of Potash", in which he uses magnesium oxide for the precipitation of phosphoric acid. This work is along the same line as that done by the Referee on Potash, who, however, used magnesium chloride for the precipitation of phosphoric acid. It would appear from these two papers that there is presented a solution of a problem that has annoyed some of the members of the association for many years, *viz.*, the fact that the full amount of potash in mixed fertilizers, especially mixtures of acid phosphate and potash salts, is not shown by the Lindo-Gladding method. The members of the association who were present last year will recall that the general referee laid special stress on this point at the close of his paper on the Lindo-Gladding method. It would appear from both of these papers that the precipitation of phosphoric acid either by magnesium oxide or by magnesium chloride will enable the analysts that use this simple modification to get the full amount of potash in mixed fertilizers by the Lindo-Gladding method without the use of any hydrochloric acid, which could not be used because the State Fertilizer Laws specify water-soluble potash. The general referee, therefore, would suggest and earnestly recommend that the Referee on Potash for next year be charged with a careful study of these two proposed modifications of the Lindo-Gladding method.

The general referee for 1924 sincerely regrets that owing to sickness since last June he has not sufficiently recovered his strength to venture to attend this, the Fortieth Annual Convention of the Association of Official Agricultural Chemists. J. H. Mitchell, Professor of Chemistry

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 265.

and Research Chemist of the South Carolina Experiment Station, is representing the Clemson Agricultural College of South Carolina.

In view of the fact that the general referee has served continuously since the office was created, it seems to him that it would be highly desirable that some younger and more active member be appointed as General Referee on Fertilizers.

No separate report on phosphoric acid was given, but the following paper was submitted by the associate referee, W. H. Ross, who worked in collaboration with R. M. Jones and A. R. Merz.

### THE GRAVIMETRIC DETERMINATION OF PHOSPHORIC ACID<sup>1</sup>.

By W. H. ROSS, R. M. JONES, and A. R. MERZ (Bureau of Soils, Washington, D. C.).

At last year's meeting of this association a paper was presented by E. L. Larison<sup>2</sup> giving observations on the determination of phosphoric acid in double superphosphates. It was reported that in the analysis of ordinary acidulated phosphates that contain less than 17 per cent of  $P_2O_5$ , reasonable agreement can be obtained among chemists using the official methods, but that the differences become serious in the analysis of high-grade materials when the spread between results is increased two- or three-fold. Variation in the reactions of the solution to which the magnesia mixture is added was claimed to be one of the chief sources of error in the determination. Lowest results were obtained when the solution was made alkaline with ammonia before adding the magnesia mixture and highest when the solution was made acid. It was concluded that the latter procedure gives correct results, and it was accordingly recommended that the direction "nearly neutralize" in the official methods be changed to read, "neutralize the cooled solution with hydrochloric acid, using litmus as indicator, and then add 1 cc. of 1.18 sp. gr. hydrochloric acid".

This recommendation was supported by Caro<sup>3</sup> in a paper presented at this year's Fertilizer Division Meeting of the American Chemical Society. McCandless and Burton<sup>4</sup>, however, report at the same meeting that best results are obtained when the magnesia mixture is added to a neutral solution. It was claimed that an excess of acid gives results that are too high, while an excess of ammonia gives low results.

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<sup>1</sup> Presented by R. M. Jones.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 394.

<sup>3</sup> *Am. Fertilizer*, 1924, 61, No. 8: 22.

<sup>4</sup> *Ind. Eng. Chem.*, 1924, 16: 1267.

In view of these observations, a careful study of this phase of phosphate analysis was undertaken at the suggestion of R. N. Brackett, the General Referee on Fertilizers.

The starting material used in this work was crystallized phosphoric acid, prepared by crystallizing the ordinary C. P. phosphoric acid and repeating the crystallization three times. The crystals were then dried by allowing to stand for six months over dry phosphorus pentoxide. Their absolute freedom from moisture and all other impurities was shown by analysis, by the melting point of the crystals, and by their rate of crystallization. Use was also made of a sample of disodium phosphate that had been crystallized five times from the C. P. salt, and of two standard samples of phosphate rock prepared by the Bureau of Standards and the Bureau of Soils, respectively.

In analyzing these materials a series of four sets of determinations was run in duplicate. In the first set the solutions were made strictly neutral before adding the official magnesia mixture; in the second they were made alkaline by use of an excess of ammonia equivalent to 1 cc. of 0.90 sp. gr. ammonia; and in the third they were made acid by the addition,

TABLE 1.  
*Analysis of phosphatic materials.*

MATERIAL	PHOSPHORIC ACID ( $P_2O_5$ ) PRESENT	PHOSPHORIC ACID ( $P_2O_5$ ) FOUND			
		Reaction of solution before precipi- tating with alkaline magnesia mixture			Adding acid magnesia mixture to alkaline solution
		Alkaline	Neutral	Acid	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Crystallized phosphoric acid.....	72.44	72.12	72.52	72.83	72.53
Disodium phosphate.....	50.01	49.92	50.06	50.63	50.35
Standard phosphate rock, Bureau of Standards....	31.45*	31.51	31.56	31.70	31.48
Standard phosphate rock, Bureau of Soils.....	31.09†	30.91	31.07	31.25	31.09

\* As found by the Bureau of Standards.

† As found by the Bureau of Soils.

beyond the neutral point, of the equivalent of 1 cc. of 1.18 sp. gr. hydrochloric acid. The fourth set was treated according to the recommendations of the Bureau of Standards, acid magnesia mixture being used. The neutral point was determined in each case by the use of bromthymol blue as indicator, which was found much more convenient than litmus.

The results obtained and listed in Table 1 show slight variations, according as the solution was made alkaline, neutral, or acid preparatory to precipitating with magnesia mixture. The differences are within the limit of experimental error for a single set of determinations, but the values obtained in a large number of analyses convince the writers that the reaction of the solution does have some effect on the results, as claimed by Larison, by Caro, and by McCandless and Burton. In agreement with McCandless and Burton, it was found that the best results are obtained by use of a neutral solution, as specified in the official methods, rather than with the acid solution recommended by Larison and by Caro.

The reagents used in this work were carefully treated for the elimination of possible impurities by crystallization of the C. P. salts. This was thought to be necessary by reason of the poor quality of many analytical reagents. This applies particularly to the ammonium molybdate and magnesia mixture solutions used in phosphate analysis, and it is the opinion of the writers that greater differences in analytical results arise from the use of impure chemicals than from the slight variations in the reaction of the solutions to which the magnesia mixture is added.

It has been observed that an alkaline magnesia mixture that had been standing in a glass bottle for a time gave a precipitate with crystallized phosphoric acid that failed to burn white and that was not entirely soluble in hydrochloric acid. The precipitate obtained by use of a freshly prepared magnesia mixture burned perfectly white and left no acid-insoluble residue. This would indicate that inaccurate results may follow the use of magnesia mixture that has seriously reacted on its glass container. To lessen this possibility the Bureau of Standards has recommended the use of magnesia mixture of a slightly acid reaction.

It is possible to obtain accurate results in the determination of phosphoric acid by the official gravimetric method, but it should be emphasized that if the results obtained in the analysis of high-grade phosphates are to be correct within the same arithmetical leeway usually required for the ordinary low-grade fertilizers much greater care must be taken in the manipulation of the method than is necessary for the low-grade materials.

The writers agree with those who maintain that more specific directions should be given in the official methods for the determination of phosphoric acid and therefore recommend that such a collaborative study be made of the subject next year as will include the use of acid magnesia mixture with a view to its possible substitution for the alkaline mixture of the present official method.

REPORT ON NITROGEN<sup>1</sup>.

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*.

The work of the associate referee for the past year has been in accordance with the plans formulated in the report on nitrogen made at the last annual meeting of the association<sup>2</sup>. This consisted of a study of the Devarda<sup>3</sup> and Moore-Kjeldahl<sup>4</sup> methods for the determination of nitrates in the nitrates of commerce. The use of sodium thiosulfate as a substitute for sodium or potassium sulfide in precipitating mercury in the Moore-Kjeldahl method was also studied.

Three commercial samples of nitrate were carefully prepared and sent to twenty-five chemists who had signified their willingness to cooperate. Devarda alloy (20-mesh) for conducting the work was also supplied the collaborators. Twenty-two reports were received.

## INSTRUCTIONS TO COLLABORATORS.

## REAGENTS FOR DEVARDA ALLOY METHOD.

- (a) *Devarda alloy*.
- (b) *0.2 N standard direct acid*.
- (c) *0.1 N standard direct alkali*.
- (d) *Methyl red indicator*.—Dissolve 0.02 grams in 100 cc. of hot water (10 drops for each titration).

## DETERMINATION.

Weigh directly from the sample bottles 10 grams of the nitrate salt and make up exactly to 500 cc. Use 25 cc. aliquots for each determination.

Place 25 cc. of the nitrate solution in a 500–700 cc. flask and add 300 cc. of water, 3 grams of the Devarda alloy, and 5 cc. of sodium hydroxide solution (42 per cent by weight, sp. gr. 1.453), pouring the sodium hydroxide down the side of the flask so that it does not mix at once with the contents. Connect by means of a Davison scrubber<sup>5</sup>, or other suitable scrubbing bulb that will prevent the passing over of any portion of the spray, with a condenser, the tip of which condenser always extends beneath the surface of the standard acid in the receiving flask. Mix the contents of the distilling flask by rotating. Heat slowly at first, and then at such a rate that 250 cc. of the distillate will pass over in 1 hour. Collect the 250 cc. distillate in a measured quantity of standard acid and titrate with standard alkali solution, using methyl red as indicator.

## REAGENTS FOR THE MOORE-KJELDAHL METHOD.

(a) *Salicyl-sulfonic acid*.—40 grams of salicylic acid is made up to 1 liter with concentrated sulfuric acid.

(b) *Sodium thiosulfate (hyposulfite) (hypo)*,  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ .—Commercial photographic, pea size.

<sup>1</sup> Paper No. 243 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 381.

<sup>3</sup> *J. anal. Chem.*, 1894, 33: 113; H. W. Wiley. Principles and Practices of Agricultural Analyses, 1908, 2: 428; *J. Assoc. Official Agr. Chemists*, 1922, 5: 451.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1920, 12: 669.

<sup>5</sup> *Ibid.*, 1919, 11: 465.

(c) *Potassium or sodium sulfate*.—Preferably dry powder.

(d) *Mercuric oxide*.

(e) *Caustic soda*.—Dissolve 30 pounds of commercial caustic soda in about 2.5 gallons of water, let settle, and siphon off the clear solution. This strong caustic soda is practically free from carbonate.

(f) *Sodium sulfide*.—Dissolve 100 grams of fused sodium sulfide in water and dilute to 1000 cc.

(g) *Pure granulated, or 20- or 30-mesh zinc*.—Pure zinc is essential as impure zinc reacts so actively with the sodium hydroxide that the rapid evolution of hydrogen carries over by entrainment some free alkali, even when using the Hopkins connecting bulb. This causes a variable blank. The Davison bulb will prevent this entrainment.

(h) *0.5 N sulfuric acid solution*.

(i) *0.2 N or 0.1 N sodium hydroxide solution*.

(j) *Sodium alizarin sulfonate*.—2 grams in 100 cc. of water.

#### DETERMINATION.

Transfer 25 cc. of the nitrate solution, preferably, to a 650 cc. Pyrex Kjeldahl flask. Place the flask on a steam or hot water bath and evaporate to dryness. Add 35 cc. of salicyl-sulfonic acid, preferably from a dispensing buret, rinsing down the neck of the flask; warm over low heat or in boiling water or steam bath until reaction begins, shaking frequently until solution is complete. Do not heat the flask hotter than the hand can bear, otherwise loss of nitrogen may occur. Add 5 grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  hypo, heat over low heat until frothing ceases (about 5 minutes), then add 10 grams of sodium or potassium sulfate and 1 gram of mercury, and continue digestion until clear, and for one hour afterwards, boiling briskly; cool; dilute with water to about 400 cc., and add a small pinch of 20- or 30-mesh zinc (0.1 gram) and 70 to 80 cc. of caustic soda in which are dissolved 2 grams of fused sodium sulfide or potassium sulfide. Sulfide may be added before adding the zinc and sodium hydroxide. The ammonia is distilled and collected in 0.5 N sulfuric or hydrochloric acid. About 200–250 cc. distillates are sufficient, requiring about  $\frac{1}{2}$  to  $\frac{3}{4}$  hour. Use in receiving flask a sufficient quantity of 0.5 N acid diluted to 75–100 cc. with distilled water and three drops of sodium alizarin sulfonate.

*Alternative indicator*.—A solution of cochineal is prepared by digesting and frequently agitating 3 grams of pulverized cochineal in a mixture of 50 cc. of strong alcohol and 200 cc. of distilled water for a day or two at ordinary temperatures. Five cc. of the filtered solution is employed as an indicator. This cochineal solution will keep in good condition for use for about two weeks only.

#### EXPERIMENTS.

*Series I*.—Blanks for Devarda alloy method. Prepare the reagents and conduct three determinations of the nitrogen in the reagents used, following the directions given. Record the results on the blank sheets prepared under Series I. (Do not round off the figures or give averages.)

*Series II*.—Dissolve exactly 10 grams of the sodium nitrate sample in 500 cc. of distilled water. Use 25 cc. portions for each determination by the Devarda method. Conduct three determinations.

*Series II-A*.—Repeat Series II with the potassium nitrate sample.

*Series II-B*.—Repeat Series II with the nitrapo (high potash nitrate) sample.

*Series III*.—Run three blanks on the reagents for the Moore-Kjeldahl method.

*Series IV*.—Accurately transfer by means of the same pipet used in the Devarda alloy work 25 cc. portions of the sodium nitrate sample to a 650 cc. Pyrex flask, place flask on a steam or hot water bath, evaporate to dryness, and proceed according to the directions for the Moore method. Conduct three determinations.



*Series IV-A.*—Repeat Series IV with the potassium nitrate sample.

*Series IV-B.*—Repeat Series IV with the nitrapo (high potash nitrate) sample.

*Series V.*—Repeat Series IV, Series IV-A, and Series IV-B above, except that the mercury is to be precipitated in these experiments with 2 grams of sodium thiosulfate,  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , dissolved in 25 cc. of distilled water.

It is suggested that Pyrex glass apparatus be used throughout. If any departure is made from the above procedure in any particular, please note the fact. The scrubbing bulb should contain 20–30 cc. of water during the distillation. The standard acid and alkali may be of a different strength from that indicated in the procedures, but please state the strength of the solution used.

The collaborators were as follows:

1. N. F. Ramsey, Picatinny Arsenal, Dover, N. J.
2. C. M. Bible, Read Phosphate Co., Nashville, Tenn.
3. A. O. Olson, Dairy and Feed Commission, St. Paul, Minn.
4. W. D. Richardson, Swift & Co., Chicago, Ill.
5. T. L. Roettgers, Southern Cotton Oil Co., Savannah, Ga.
6. B. L. Caldwell, Southern Cotton Oil Co., Savannah, Ga.
7. L. D. Haigh, University of Missouri, Columbia, Mo.
8. W. R. Austin, Tennessee Chemical Co., Nashville, Tenn.

TABLE 1.  
*Devarda alloy method.*  
(Expressed as percentage of nitrogen.)

COLLABORATORS	SERIES II			SERIES II-A			SERIES II-B		
	Sodium nitrate			Potassium nitrate			High potash nitrate		
	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average
1	15.64	15.54	15.60	12.75	12.68	12.70	15.42	15.24	15.31
2	15.62	15.54	15.59	12.58	12.54	12.57	15.18	15.18	15.18
3	15.71	15.65	15.69	12.54	12.48	12.51	15.17	15.14	15.15
4	15.63	15.60	15.62	12.51	12.50	12.51	15.18	15.17	15.18
5	15.35	13.66	14.68	11.35	10.46	10.93	13.92	13.54	13.69
6	12.65	12.55	12.60	10.79	10.62	10.72	12.67	12.53	12.58
7	15.51	15.26	15.41	12.43	12.28	12.36	15.05	14.94	14.98
8	15.66	15.66	15.66	12.50	12.50	12.50	15.32	15.32	15.32
9	15.70	15.36	15.48	12.42	12.32	12.36	15.19	14.91	15.10
10	15.69	15.66	15.68	12.60	12.56	12.58	15.25	15.19	15.23
11	15.70	15.62	15.65	12.56	12.44	12.51	15.20	15.20	15.20
12	15.61	15.58	15.59	12.59	12.58	12.58	15.10	15.04	15.08
13*	15.38	15.05	15.22	12.16	12.08	12.12	14.78	14.49	14.63
14	14.90	14.82	14.85	12.02	12.00	12.01	14.74	14.70	14.72
15	15.57	15.54	15.56	12.54	12.45	12.51	15.18	15.15	15.17
16	15.70	15.66	15.69	12.64	12.56	12.59	15.20	15.10	15.17
17	15.54	15.42	15.50	12.58	12.42	12.49	15.18	15.02	15.09
18	15.67	15.35	15.54	12.42	12.22	12.35	14.83	14.74	14.80
19	15.82	15.78	15.79	12.62	12.62	12.62	15.34	15.30	15.33
20	15.22	15.18	15.20	12.48	12.60	12.53	15.17	15.12	15.15
21	15.73	15.60	15.68	12.70	12.60	12.64	15.34	15.23	15.30
22	15.99	15.95	15.97	12.81	12.79	12.80	15.55	15.47	15.51

	HIGHEST	LOWEST	AVERAGE
* Series II using usual scrubber.....	15.30	15.24	15.27
Series II zinc iron method.....	15.27	15.05	15.16

9. L. W. Tarr, University of Delaware, Newark, Del.
10. H. C. McLean, Experiment Station, New Brunswick, N. J.
11. R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
12. C. D. Garby, Fixed Nitrogen Research Laboratory, Washington, D. C.
13. L. S. Walker, Massachusetts Agricultural Experiment Station, Amherst, Mass.
14. W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.
15. H. L. Moxon, Virginia-Carolina Chemical Co., Richmond, Va.
16. O. B. Winter, Agricultural College, East Lansing, Mich.
17. T. J. Kuhlmann, Jr., Department of Agriculture, Harrisburg, Pa.
18. J. J. Morris, Mississippi Agricultural and Mechanical College, Agricultural College, Miss.
19. L. A. Salinger, Bureau of Chemistry, Savannah, Ga.
20. Victor Conquest, Armour & Company, Chicago, Ill.
21. R. L. Jones and J. B. Smith, Agricultural Experiment Station, Kingston, R. I.
22. T. H. Hopper, North Dakota Agricultural College, Agricultural College, N. Dak.

Tables 1, 2, and 3 give the results obtained by the collaborators, and Table 4 gives the average result for each series by each collaborator. In Table 5 a summary of the collaborative results is prepared.

TABLE 2.  
*Moore-Kjeldahl method.*  
(Expressed as percentage of nitrogen)

COLLABORATORS	SERIES IV			SERIES IV-A			SERIES IV-B		
	Sodium nitrate			Potassium nitrate			High potash nitrate		
	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average
1	15.66	15.48	15.55	12.46	12.38	12.41	15.32	15.18	15.23
2	15.66	15.62	15.65	12.62	12.54	12.56	15.26	15.18	15.21
3	15.66	15.53	15.60	12.59	12.39	12.50	15.12	14.38	14.69
4	15.60	15.58	15.59	12.59	12.56	12.57	15.20	15.19	15.19
5	15.66	15.19	15.44	12.55	12.27	12.40	15.10	14.97	15.01
6	15.46	15.37	15.41	12.38	12.35	12.36	14.98	14.84	14.93
7	15.45	14.92	15.25	12.43	12.10	12.23	15.02	14.27	14.75
8	15.72	15.66	15.70	12.50	12.46	12.49	15.26	15.22	15.23
9	15.79	15.64	15.74	12.51	12.39	12.46	15.43	15.31	15.35
10	15.61	15.54	15.58	12.52	12.40	12.49	15.13	15.06	15.09
11	15.62	15.58	15.59	12.46	12.40	12.43	15.20	15.16	15.19
12	15.61	15.58	15.60	12.53	12.49	12.50	15.06	15.03	15.05
13	15.52	15.03	15.28	12.11	11.78	11.95	14.84	14.06	14.45
14	14.84	14.72	14.79	12.08	11.94	12.01	14.80	14.60	14.69
15	15.54	15.40	15.49	12.52	12.45	12.50	15.18	15.12	15.15
16	15.66	15.44	15.57	12.56	12.56	12.56	15.16	15.06	15.12
17	15.70	15.59	15.63	12.43	12.35	12.40	15.24	15.16	15.19
18	15.88	15.74	15.83	12.63	12.55	12.58	15.10	15.01	15.07
19	15.82	15.78	15.81	12.64	12.62	12.63	15.34	15.28	15.31
20	15.73	15.72	15.72	12.58	12.58	12.58	15.16	15.16	15.16
21	15.67	15.54	15.60	12.60	12.50	12.55	15.22	15.15	15.18
22	15.82	15.74	15.79	12.68	12.66	12.67	15.28	15.26	15.27

TABLE 3.

*Moore-Kjeldahl method (using  $\text{Na}_2\text{S}_2\text{O}_8 \cdot 5\text{H}_2\text{O}$  to precipitate Hg).*

(Expressed as percentage of nitrogen.)

COLLABORATORS	SERIES V			SERIES V-A			SERIES V-B		
	Sodium nitrate			Potassium nitrate			High potash nitrate		
	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest	Average
1	15.54	15.46	15.51	12.52	12.42	12.45	15.38	15.24	15.32
2	15.58	15.50	15.54	12.54	12.50	12.53	15.14	15.10	15.13
3	15.53	15.40	15.48	12.43	12.36	12.38	15.24	14.92	15.05
4	15.66	15.63	15.64	12.58	12.54	12.56	15.21	15.18	15.20
5	15.52	15.33	15.41	12.50	12.30	12.38	15.06	14.91	14.97
6	15.46	15.37	15.40	12.40	12.32	12.37	15.03	14.90	14.98
7	15.51	15.10	15.33	12.35	11.82	12.16	15.00	14.90	14.95
8	15.68	15.66	15.67	12.50	12.46	12.49	15.18	15.18	15.18
9	15.76	15.65	15.72	12.63	12.48	12.55	15.29	15.19	15.25
10	15.48	15.48	15.48	12.43	12.41	12.42	15.16	15.11	15.13
11	15.70	15.64	15.66	12.46	12.32	12.39	15.12	15.10	15.11
12	15.39	15.36	15.38	12.44	12.33	12.40	15.10	15.06	15.07
13	...	...	...	...	...	...	...	...	...
14	...	...	...	...	...	...	...	...	...
15	15.54	15.48	15.52	12.52	12.46	12.48	15.09	15.06	15.08
16	15.66	15.64	15.65	12.58	12.52	12.55	15.20	15.14	15.17
17	15.80	15.76	15.78	12.54	12.44	12.49	15.38	15.35	15.36
18	15.80	15.77	15.78	12.66	12.66	12.66	15.35	15.10	15.27
19	15.84	15.82	15.83	12.62	12.58	12.61	15.34	15.30	15.33
20	15.68	14.84	15.33	12.50	12.48	12.49	15.19	15.12	15.15
21	15.78	15.66	15.71	12.62	12.52	12.57	15.38	15.28	15.33
22	15.78	15.38	15.64	12.78	12.64	12.69	15.28	15.26	15.27

## NOTES ON THE DEVARDA ALLOY METHOD.

The alloy used in the Devarda method consists of aluminium (45 per cent), copper (50 per cent), and zinc (5 per cent)<sup>1</sup>. The strength of the alkali is of importance. If too strong, the action of the alloy is unduly vigorous at the beginning. If it is too weak, the contents of the flask have to be overheated, the result in either case causing the formation of a fine spray of caustic solution. With the right strength of alkali (42 per cent by weight, sp. gr. 1.453) the spray produced is at a minimum, and the use of a Davisson scrubber absorbs what is produced.

## COMMENTS BY ANALYSTS.

*N. F. Ramsey.*—Good checks were obtained in general with the Devarda alloy method, but the averages of the results of the various series with this method were higher in general than those obtained with the Moore-Kjeldahl method.

*Herbert S. Bailey.*—Commenting on T. L. Roettger's and B. L. Caldwell's work Bailey says: "Their results with the Devarda alloy method are far from satisfactory. Last year Roettger got very good figures on the pure salts, I believe, but apparently there is something about the products on which they have worked this year which makes it difficult to use the Devarda alloy method."

<sup>1</sup> *Chem. Ztg.*, 1892, 16: 1952.

TABLE 4.  
Averages.  
(Expressed as percentage of nitrogen.)

COLLABORATORS	SODIUM NITRATE			POTASSIUM NITRATE			HIGH POTASH NITRATE		
	Series II	Series IV	Series V	Series II-A	Series IV-A	Series V-A	Series II-B	Series IV-B	Series V-B
1	15.60	15.55	15.51	12.70	12.41	12.45	15.31	15.23	15.32
2	15.59	15.65	15.54	12.57	12.56	12.53	15.18	15.21	15.13
3	15.69	15.60	15.48	12.51	12.50	12.38	15.15	14.69	15.05
4	15.62	15.59	15.64	12.51	12.57	12.56	15.18	15.19	15.20
5	14.68	15.44	15.41	10.93	12.40	12.38	13.69	15.01	14.97
6	12.60	15.41	15.40	10.72	12.36	12.37	12.58	14.93	14.98
7	15.41	15.25	15.33	12.36	12.23	12.16	14.98	14.75	14.95
8	15.66	15.70	15.67	12.50	12.49	12.49	15.32	15.23	15.18
9	15.48	15.74	15.72	12.36	12.46	12.55	15.10	15.35	15.25
10	15.68	15.58	15.48	12.58	12.49	12.42	15.23	15.09	15.13
11	15.65	15.59	15.66	12.51	12.43	12.39	15.20	15.19	15.11
12	15.59	15.60	15.38	12.58	12.50	12.40	15.08	15.05	15.07
13	15.22	15.28	...	12.12	11.95	...	14.63	14.45	...
14	14.85	14.79	...	12.01	12.01	...	14.72	14.69	...
15	15.56	15.49	15.52	12.51	12.50	12.48	15.17	15.15	15.08
16	15.69	15.57	15.65	12.59	12.56	12.55	15.17	15.12	15.17
17	15.50	15.63	15.78	12.49	12.40	12.49	15.09	15.19	15.36
18	15.54	15.83	15.78	12.35	12.58	12.66	14.80	15.07	15.27
19	15.79	15.81	15.83	12.62	12.63	12.61	15.33	15.31	15.33
20	15.20	15.72	15.33	12.53	12.58	12.49	15.15	15.16	15.15
21	15.68	15.60	15.71	12.64	12.55	12.57	15.30	15.18	15.33
22	15.97	15.79	15.64	12.80	12.67	12.69	15.51	15.27	15.27

TABLE 5.  
Summary of collaborative results (expressed as percentage of nitrogen).

AVERAGES	SODIUM NITRATE			POTASSIUM NITRATE			HIGH POTASH NITRATE		
	De- varda method	Moore method	$\text{Na}_2\text{S}_2\text{O}_3$ to ppt the Hg	De- varda method	Moore method	$\text{Na}_2\text{S}_2\text{O}_3$ to ppt the Hg	De- varda method	Moore method	$\text{Na}_2\text{S}_2\text{O}_3$ to ppt the Hg
	Series II	Series IV	Series V	Series II-A	Series IV-A	Series V-A	Series II-B	Series IV-B	Series V-B
Total average . . . .	15.37	15.55	15.57	12.33	12.45	12.48	14.99	15.08	15.17
Average, omitting results of Collaborators 5 and 6 . . . .	15.55			12.49			15.13		

L. D. Haigh.—The tendency for the solution in the distilling flask to foam during the distillation is marked in the Devarda alloy method. Some help may be obtained by adding to each flask a piece of paraffin about the size of a large pea.

On the Moore method Haigh comments: "The distilling bulb used was the Davisson scrubbing bulb, the same that was used on the Devarda alloy method. My observation is, that its use on the distillation of the Moore method is not so essential as for the Devarda alloy method. The amount of alkalinity found in the water in the bulb after the Moore distillation is very small, if any; after the distillation from the Devarda alloy method an appreciable quantity is generally found".

*W. R. Austin.*—We made a number of determinations in addition to those asked for by you, along the line of last year's work, and found very little difference in results. In a few instances those weighed out directly (0.5 gram portions) were equivalent to approximately 0.05 per cent higher. This, however, I attribute entirely to weighing.

*L. S. Walker.*—Results by Moore's method very erratic. (Walker tried a modification of the Devarda method by using the usual Kjeldahl attachment or scrubber in place of the Davisson scrubber.) Results secured would indicate that the usual scrubber used in the ordinary Kjeldahl work is quite as effective in the Devarda method as is the Davisson scrubber. (Duplicate determinations made on the nitrate of soda solution, using the zinc-iron method<sup>1</sup> indicate that this method gives approximately the same results as the Devarda alloy method.)

*R. L. Jones and J. B. Smith.*—A scrubber proved a necessity for the Devarda method and a further benefit was noted in that the boiling was more steady with less frothing.

Commenting on the Moore method they say: "Preliminary tests showed that our large Kjeldahl bulbs were as efficient as scrubbers, and the latter were not used for this method".

*T. H. Hopper.*—The results with the Devarda alloy method are the highest in all cases. This may be due to a loss of nitrogen on adding the salicyl-sulfonic acid to the dried residues. A slight odor of nitric acid was noticed after adding the acid to the potassium nitrate residues.

#### DISCUSSION AND CONCLUSIONS.

The majority of analysts found no difficulty with the Devarda alloy method. However, Collaborators 5 and 6 of the same laboratory had some special difficulty with the method this year, causing low results. Consequently, in studying the general averages by this method as shown in Table 5, it is best to omit results of Collaborators 5 and 6. Otherwise the figures will be misleading.

The results secured by a large number of collaborators using the Devarda alloy method on commercial samples of nitrates indicate that the method is quite satisfactory. Previous collaborative work done in 1921 and again in 1922 showed that the method was satisfactory for pure nitrates. The simplicity of the method, together with the consistent and concordant results obtained among the majority of collaborators would suggest the adoption of this method as official.

The results obtained by the Moore-Kjeldahl method tended to run slightly lower than those obtained by the Devarda alloy method in the majority of cases. This fact was also noticed in the collaborative work done in 1922. In the hands of many chemists this method seems to be entirely satisfactory. However, there are a number of points in the procedure that require careful attention, and probably this accounts for some of the variation in results obtained by collaborators. Besides, it is the opinion of the associate referee that a shorter and less expensive method is to be preferred where the nitrogen in nitrate salts is the only form of nitrogen to be determined. This method would probably be

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 669.

very satisfactory where organic nitrogen is to be determined along with nitrate nitrogen.

The use of sodium thiosulfate as a precipitant for mercury in the Kjeldahl or Moore-Kjeldahl method appears to be quite satisfactory, judged from the results of the collaborators this year. The differences in results between this method and where potassium sulfide is used to precipitate the mercury seem to be slight and of no significance. This can readily be seen from Table 4 and especially in Table 5 where the total average is reported. The associate referee believes that the use of sodium thiosulfate for the above purpose should be left to the discretion of the analyst.

#### RECOMMENDATIONS.

It is recommended—

(1) That the Devarda alloy method as given in this report be adopted as a tentative method to determine nitrogen in nitrate salts.

(2) That the use of sodium thiosulfate instead of potassium or sodium sulfide to precipitate the mercury in the Kjeldahl method and its modifications be adopted as an optional procedure.

#### THE DETERMINATION OF AVAILABLE NITROGEN IN MIXED FERTILIZERS BY THE OFFICIAL NEUTRAL PERMANGANATE METHOD AS USED IN FLORIDA.

By GORDON HART (Department of Agriculture, Tallahassee, Fla.).

Available nitrogen may be defined, when speaking of fertilizers, as nitrogen that is available to plants when applied to them as a fertilizer, or as nitrogen that can be taken up from the soil by plants and made a part of the plant. It may also be available at the time of application or become so during the growing season or following seasons. Nitrate and ammonia salts are in the first class, organic nitrogen is in the second class, and some nitrogen is scarcely available at all.

The separation of the available organic nitrogen from that which is not available to the plant is the subject of this discussion.

The fertilizer law of Florida requires that fertilizer sold in the State shall be guaranteed in available and insoluble ammonia and that the State Chemist make these determinations. The law also requires that the analyses be made by the methods of the Association of Official Agricultural Chemists. However, the methods do not define available nitrogen; neither do they give any way for determining it. They do give the neutral permanganate and the alkaline permanganate methods for the classification of organic nitrogen as good or bad. These methods do not produce results giving the percentage of good or bad organic nitrogen; they either pass all water-insoluble organic nitrogen, or condemn it all.

At the meeting in November, 1923, R. E. Rose, the State chemist, and the writer requested that the association adopt a method for the determination of available nitrogen. This was refused on the grounds that at present there was available no method of chemical analysis that was considered sufficiently dependable for this determination.

The adoption of a method for the State of Florida was then taken up with the State chemists. The neutral permanganate method with some changes was adopted. While it is not considered that this method is infallible, under the circumstances it appears to be the best at present. The authorities are looking for a better method and will be glad to adopt any that is proved to be correct.

The neutral permanganate method for insoluble organic nitrogen, with one change, has been adopted. A one gram sample is used for the determination of available nitrogen in mixed fertilizers instead of a portion equivalent to 50 milligrams of water-insoluble nitrogen. This change was made for the following reasons:

If a portion equivalent to 50 milligrams of water-insoluble organic nitrogen is used, the sample ranges in size from one-half to ten grams of fertilizer. This is a very wide range when one is subject to referee work and especially as each chemist makes his own determination of water-insoluble nitrogen. One laboratory could easily vary from another as much as one gram on the sample used, owing to different room temperature and the personal equation of the chemists themselves. Therefore, for the enforcement of the law, it is considered that a portion of fertilizer equivalent to 50 milligrams of water-insoluble organic nitrogen is too indefinite.

In the second place, from a study of twelve samples of mixed fertilizer made by the writer<sup>1</sup>, it was shown that less than one gram sample with the 0.5 *N* standard solution generally used in a fertilizer laboratory gave too wide a variation, due to probable errors in analysis. In other words, when less than one gram was used, good checks could not be obtained. On the other hand, as the size of the sample was increased, the checks became much closer, but after passing three grams, the results commenced to show that the permanganate was all used up, and that the solutions were losing their color. The writer also made determinations on several samples of fertilizer materials to see if the water-insoluble phosphoric acid was acted on by the neutral permanganate. The results showed that it was.

From this study it would appear that the portion of fertilizer equivalent to 50 milligrams of water-insoluble nitrogen was not entirely correct. It also appeared best for compliance with the Florida law to use a sample as small as possible to be accurate.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 379.

One feature of the determination as now made in this laboratory is not desirable. It does not seem possible to make an accurate transfer of a fertilizer sample from a filter paper on which it has been washed. It is found that by fast washing and transferring at once after the last washing is completed most of the fertilizer can be washed off with the 25 cc. of water allowed for transferring. Checks on the insoluble nitrogen show that what is lost does not greatly interfere with the accuracy of the determination.

Out of some 300 samples analyzed by this method, very few have shown any considerable quantity of insoluble nitrogen. A few have shown as much as 0.40 per cent. Most of the determinations show less than 0.10 per cent of insoluble nitrogen present.

When it is considered that the average growing season in the South for most field crops, except cotton, is but little over three months, it does not seem that much of the nitrogen that shows insolubility by the neutral permanganate method as used in this laboratory can be available in that time. However, in Florida many fruit trees grow the year round. The climate is also so mild that the winter does not stop bacterial action. It is the opinion of the writer that if the insoluble nitrogen has been ground fine enough, it will become available to these fruit trees before more than two or three years have passed.

In conclusion, doubt may be expressed as to whether the determination of available nitrogen as reported under the Florida law is of any great value.

## REPORT ON POTASH.

By A. P. KERR (Agricultural Experiment Station, Baton Rouge, La.),  
*Associate Referee.*

Most of the reasons advanced for inability to determine the total amount of potash in fertilizers by the A. O. A. C. water-soluble method have had something to do with the precipitate potassium chloroplatinate, either that it was an impure precipitate or that the strength of alcohol washing affected it. Moore and Caldwell<sup>1</sup> reported that 80 per cent alcohol did dissolve some of the chloroplatinate and that 95 per cent alcohol should be used instead. R. N. Brackett, the General Referee on Fertilizers, after reviewing the literature on the subject, seemed to think that this statement was not according to the facts. Hazen has also reported results<sup>2</sup>.

Work was carried out by the writer in the Louisiana Experiment Station laboratory during the last few months to ascertain whether or not the water-insoluble method did give low results on all mixed fertilizers. The

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 1188.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 456.



conclusion drawn from this work was that it depended largely on the kind of fertilizer. Combinations of acid phosphate and sulfate of potash and acid phosphate and muriate of potash were thoroughly mixed and analyzed by the A. O. A. C. method, and results that agreed very closely with the quantities of potash used were obtained, but when a small quantity of phosphoric acid was added the results obtained were from about 0.2 to 0.3 per cent too low.

Complete fertilizers, as found on the market, were than studied in regard to the contents of the solution after ammonium hydroxide and ammonium oxalate had been added. It was found that in these fertilizers this solution contained either phosphorus (probably from  $H_2PO_4$ ) or magnesium salts in solution.

The results with the samples that contained phosphorus were low in most every determination, whereas the results with the samples that contained magnesium salts in solution would agree closely with the analyses made with a 1 per cent hydrochloric acid solution. All samples digested with a 1 per cent hydrochloric acid solution contained magnesium salts and possibly a trace of phosphorus.

Samples that contained phosphorus after ammonium hydroxide had been added were then studied, the object being to remove the phosphorus. Very good results were obtained by adding 25 cc. of magnesium chloride solution (1 gram to 100 cc. of water) to the sample after boiling 30 minutes and just before adding ammonium hydroxide and ammonium oxalate<sup>1</sup>.

## A MODIFICATION OF THE OFFICIAL LINDO-GLADDING METHOD FOR THE DETERMINATION OF POTASH.

By C. M. BIBLE (Read Phosphate Co., Nashville, Tenn.).

The reports of investigators using the official Lindo-Gladding method for the determination of potash in mixtures of acid phosphate and potash salts show the failure of that method to get theoretical results. The writer has had similar experiences, when working on mixtures of known composition, particularly in the case of mixtures of acid phosphate and potash salts.

In such mixtures, after the potash has been extracted with boiling water and precipitation made with ammonia and ammonium oxalate, there is usually much phosphoric acid remaining in the filtrate. It is the writer's belief that the phosphoric acid in the filtrate interferes with the accuracy of the potash determination. Observations by the writer have been that mixtures that gave filtrates low in phosphoric acid have not caused much difficulty, but when the filtrates contained much phosphoric acid low results were obtained.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 263.

When filtrates high in phosphoric acid are evaporated and the residues ignited in porcelain or silica dishes and then dissolved in water or water acidified with hydrochloric acid, there remains much insoluble material that must be removed by filtration before the addition of platinum solution. Even with the insoluble matter filtered out in this way, in many cases siliceous matter contaminates the final precipitate. In working on mixtures containing treble superphosphate, the writer could not finish the determinations at all in silica dishes, and when platinum dishes were used there was considerable attack on the dishes and the results were low.

To determine whether or not the low results were due mostly to the phosphoric acid present after precipitation with ammonia and ammonium oxalate, the writer has tried out the following modification of the official Lindo-Gladding method:

After extraction of the potash with boiling water and precipitation with ammonia and ammonium oxalate, sufficient powdered magnesium oxide is added to precipitate all the phosphoric acid. The flask is cooled, made to volume, and the solution is filtered and finished as in the official method without the removal of the magnesium used in excess of that required to precipitate the phosphoric acid.

The results obtained have been very promising. Mixtures high in phosphoric acid by the modified method gave theoretical results in porcelain, silica, and platinum dishes. Upon ignition there was no perceptible attack on the dishes, and the residues were very readily soluble in water and free from silica. There was no need to filter the solution before adding platinum solution.

Various other bases have been used by investigators for the removal of phosphoric acid in potash determinations. The official alternative method provides for the phosphoric acid removal, but that method is long, and the results tend to be low, owing, probably, to occlusion of potash by the heavy precipitates that result. By the use of magnesium oxide for the removal of phosphoric acid, the soluble sulfates remain in solution, and the magnesium used in excess need not be removed before adding platinum solution. Occlusion of potash by precipitates, therefore, is kept to a minimum.

In testing the modification of the official Lindo-Gladding method, a solution of potassium sulfate and superphosphate was made, so that 50 cc. of the solution would have a theoretical potash content of 0.0506 gram. Determinations were made in porcelain and silica by both the official and modified methods. The following results were obtained:

DISH	OFFICIAL LINDO-GLADDING METHOD	MODIFIED LINDO-GLADDING METHOD
	gram	gram
Porcelain . . . . .	0.0473, 0.0479	0.0506
Silica . . . . .	0.0494, 0.0494	0.0503

To make further study, three tankage-phosphate mixtures with different water-soluble phosphoric acid content were made, and the potash content was determined in each case. Then sufficient sulfate of potash of known composition was added to each mixture to make the theoretical potash content 5 per cent. The phosphoric acid remaining in the filtrates after precipitation with ammonia and ammonium oxalate was as follows:

Mixture I.....	0.0128 gram (in 50 cc. filtrate)
Mixture II.....	0.0420 gram (in 50 cc. filtrate)
Mixture III.....	0.1344 gram (in 50 cc. filtrate)

Determinations of potash were carried out in porcelain, silica, and platinum, and the following results were obtained:

DISH	OFFICIAL LINDO-GLADDING METHOD	MODIFIED LINDO-GLADDING METHOD
<i>Mixture I:</i>	<i>per cent</i>	<i>per cent</i>
Porcelain.....	4.75	5.03
Silica.....	4.92	4.99
<i>Mixture II:</i>		
Porcelain.....	4.47	5.03
Silica.....	4.88	4.99
Platinum.....	4.97	5.08
<i>Mixture III:</i>		
Porcelain.....	3.53	5.12
Silica.....	5.05*	5.00
Platinum.....	4.68†	4.90

\* Precipitate contaminated with silica.

† Platinum dish badly attacked.

Another mixture, of such phosphoric acid content that after precipitation with ammonia and ammonium oxalate 0.0304 gram of phosphoric acid remained in 50 cc. of the filtrate, was used to make a comparison of the official alternative, official Lindo-Gladding, and modified Lindo-Gladding methods. The results were as follows:

OFFICIAL ALTERNATIVE METHOD	OFFICIAL LINDO-GLADDING METHOD	MODIFIED LINDO-GLADDING METHOD
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2.77	2.84	2.93
2.76	2.87	2.89
2.78	2.84	2.89
Average 2.77	2.85	2.90

A 24-4-4 mixture containing treble superphosphate, which gave a filtrate high in phosphoric acid after precipitation with ammonia and ammonium oxalate, could not be finished in silica dishes by the official method, but gave the following results by the modified Lindo-Gladding and the official alternative methods:

MODIFIED LINDO-GLADDING METHOD	OFFICIAL ALTERNATIVE METHOD
<i>per cent</i>	<i>per cent</i>
4.18	3.83
4.00	3.96
4.08	3.89
Average 4.09	3.89

In making a comparison of the official and modified Lindo-Gladding methods on a large number of samples of mixtures made in the factory, it was found that the modified method gave results averaging 0.19 per cent higher than the official method. The results by the modified method were nearer the theoretical results that should have been obtained from the use of potash salts of known composition.

The writer believes the results obtained by the modification of the official Lindo-Gladding method warrant an investigation of that modified method by the A. O. A. C.

No report on plants was made by the referee.

## CONTRIBUTED PAPERS.

### TRIERS FOR SAMPLING FLOUR.

By H. E. ROETHE (U. S. Food and Drug Inspection Station, New York, N. Y.).

#### INTRODUCTION.

Sampling flour by a trier involves some interesting problems. The accuracy and shape of the trier, the size of the core, and the ease and rapidity of operation are important in this connection.

The unpublished results of experiments conducted by others in the Bureau of Chemistry indicate that after flour has been sacked and stored or transported the moisture content of the flour near the outer surface differs from that at the center of the sack, the intermediate zones showing smaller variations. If the moisture content of flour in a sack were uniform throughout, the drawing of a representative sample would be simple. Since it is not uniform, however, a trier, which will remove a core consisting of proportionate quantities of flour from the zones extending from the outside to the center of the sack, should be used. The quantity to be taken from each zone will be determined by the percentage volume of the entire sack represented in each zone.

This paper is based on the proposition that a sample of sacked flour which is representative of the flour at the time of sampling is to be taken. It gives determinations of the cubical contents of a 140 pound jute sack of flour, of the percentage volume in 1 inch and  $1\frac{1}{2}$  inch zones or shells of such a sack from the outside to the center, of the size of core and accuracy of the No. 4 Jabez Burns & Sons conical trier designed for cocoa bean sampling, and of the 30 inch tubular flour trier commonly used, as well as calculations on the design of proposed conical and tubular flour triers for the removal of representative cores.

#### DIMENSIONS OF A BODY OF REGULAR SHAPE WITH STRAIGHT SIDES AND ELLIPTICAL BASES EQUIVALENT TO A 140 POUND SACK OF FLOUR.

Before the volume of a 140 pound sack of flour and of 1 inch and  $1\frac{1}{2}$  inch shells thereof can be determined, the dimensions of a body with straight sides and elliptical bases, which is comparable and approximately equivalent in cubical contents to such a 140 pound sack of flour, must be calculated.

Based on numerous dimensions, the average circumference and the height of a 140 pound sack of flour was found to be 4 feet  $\frac{3}{4}$  inches and 2 feet  $1\frac{1}{2}$  inches. The average transverse or major axis of the bases was found to be 1 foot  $3\frac{1}{2}$  inches and the conjugate or minor axis, 1 foot  $1\frac{1}{2}$  inches.

Using the formula:

$$\text{Approximate circumference of ellipse} = 3.1416 \sqrt{\frac{D^2 + d^2}{2}},$$

in which  $D$  = transverse axis and  $d$  = conjugate axis, an ellipse (similar to the elliptical bases of the sack), with a circumference of 4 feet  $\frac{3}{4}$  inches, may have a transverse axis of practically 1 foot  $4\frac{1}{2}$  inches and a conjugate axis of 1 foot  $2\frac{1}{2}$  inches. A 140 pound sack of flour, therefore, is approximately equivalent to a regular body with straight sides, 2 feet  $1\frac{1}{2}$  inches high, each elliptical base of which may have a transverse axis of 1 foot  $4\frac{1}{2}$  inches and a conjugate axis of 1 foot  $2\frac{1}{2}$  inches.

#### VOLUME OF BODY EQUIVALENT TO 140 POUND SACK OF FLOUR.

Area of an ellipse = product of its axes  $\times 0.785 +$ . Therefore, the volume of a body equivalent to a 140 pound sack of flour is equivalent to (area of base  $\times$  altitude)  $16.5'' \times 14.5'' \times 25.5'' \times 0.785 = 4790$  cubic inches, in round numbers.

#### VOLUME OF 1 INCH SHELLS FROM OUTSIDE TO CENTER OF SACKS.

SHELL		VOLUME cu. in.	VOLUME OF BODY per cent
1	$14.5'' \times 12.5'' \times 23.5'' \times 0.785 = 3344$ cu. in.	4790-3344 = 1446	30.18
2	$12.5'' \times 10.5'' \times 21.5'' \times 0.785 = 2218$ cu. in.	3344-2218 = 1126	23.50
3	$10.5'' \times 8.5'' \times 19.5'' \times 0.785 = 1369$ cu. in.	2218-1369 = 849	17.72
4	$8.5'' \times 6.5'' \times 17.5'' \times 0.785 = 760$ cu. in.	1369-760 = 609	12.71
5	$6.5'' \times 4.5'' \times 15.5'' \times 0.785 = 357$ cu. in.	760-357 = 403	8.41
6	$4.5'' \times 2.5'' \times 13.5'' \times 0.785 = 119$ cu. in.	357-119 = 238	4.96
7	$2.5'' \times 0.5'' \times 11.5'' \times 0.785 = 11.3$ cu. in.	119-11.3 = 107.7	2.24
Central portion . . . . .		= 11.3	0.23
Total shells . . . . .		= 4790	99.95

Thus it is apparent that this body can be divided into seven 1 inch shells or zones, the first three representing a little over 71 per cent of the volume of the entire bag, and that the central portion remaining comprises only 0.23 per cent of the entire volume. This central portion, therefore, can be disregarded and only the seven 1 inch (or  $4\frac{1}{2}$  inch) zones considered in drawing a core.

#### JABEZ BURNS & SONS NO. 4 TRIER.

The Jabez Burns & Sons No. 4 trier, conical in shape, has one base  $\frac{1}{2}$  inch and the other  $1\frac{5}{8}$  inches in diameter and removes a core  $8\frac{1}{2}$  inches long. The face of the cone is sliced to a depth of practically  $\frac{1}{8}$  inch at the smaller end and  $\frac{1}{4}$  inch at the larger end. The volume of this core as found by calculation and also by experiment is practically  $3\frac{1}{4}$  cubic inches.

In using this trier for drawing a core from a 140 pound bag, it would be necessary to insert it to a depth of only 7 inches, the 7 inches of material removed representing the corresponding seven 1 inch zones of the bag.

Considering the length of core removed by this trier to be 7 inches, the smaller base is still  $\frac{1}{2}$  inch, but the larger base is  $1\frac{1}{4}$  inches. The volume of the 7 inch core and the 1 inch segments were calculated and the results checked by an experiment in which the actual quantity of material contained in the 1 inch segments was weighed and the weights obtained were converted to cubic inches. The volume of the 7 inch core is  $2\frac{3}{8}$  cubic inches.

In all the calculations on the determination of volume of the entire core and segments thereof removed by conical triers, the following formula was utilized: Volume of a frustum of a cone =  $0.2618a(b^2 + c^2 + bc)$ ; in which  $a$  = altitude,  $b$  and  $c$  = diameters of the two bases. The decrease in volume of the core owing to the fact that a portion of the face of the trier is cut away was calculated and subtracted from the volume obtained by the preceding formula, thus giving the true volume of the core removed.

By calculation the volume of the portion cut away was found by determining the area of segment of circle removed for each base of the desired segments of the core, using the rule:

$$\text{Area of segment of circle} = \frac{\text{rise of segment}}{\text{diameter}} \times \text{Corresponding area from}$$

table  $\times$  square of diameter<sup>1</sup>.

The average area of the two segments of circle for each segment of core was then calculated and multiplied by the length of the segment of core to give the desired volume. The sum of these volumes gave the entire volume cut away.

The volumes of the seven 1 inch segments of the core removed by the Jabez Burns trier follow:

SEGMENT.	VOLUME cu. in.	VOLUME, ENTIRE CORE per cent	VOLUME THAT SHOULD BE TAKEN per cent
1 (outer)	0.530	23.01	30.18
2	0.439	19.06	23.50
3	0.379	16.45	17.72
4	0.318	13.80	12.71
5	0.258	11.20	8.41
6	0.212	9.20	4.96
7 (inner)	0.167	7.25	2.24
Total core =	2.303	99.97	99.72

It is at once apparent that this trier removes too much core from the inner and too little from the outer zones.

#### VOLUME OF $1\frac{1}{2}$ INCH SHELLS FROM OUTSIDE TO CENTER OF SACK.

In order that the number of calculations might be reduced in working out the design of certain theoretical triers and in ascertaining the accuracy

<sup>1</sup> Rule and table are taken from "Areas of the Segments of a Circle", in Kent's Mechanical Engineer's Pocket Book, 9th ed., pp. 121, 122.

of the 30 inch tubular trier, the volumes of  $1\frac{3}{4}$  inch zones or shells were determined.

SHELL		VOLUME cu. in.	VOLUME OF BODY per cent
1	$13" \times 11" \times 22" \times 0.785 = 2469.6$ cu. in. $4790 - 2469.6 =$	2320.4	48.45
2	$9.5" \times 7.5" \times 18.5" \times 0.785 = 1034.7$ cu. in. $2469.6 - 1034.7 =$	1434.9	29.95
3	$6" \times 4" \times 15" \times 0.785 = 282.6$ cu. in. $1034.7 - 282.6 =$	752.1	15.69
4	$2.5" \times 0.5" \times 11.5" \times 0.785 = 11.3$ cu. in. $282.6 - 11.3 =$	271.3	5.65
5	(center) . . . . . =	11.3	0.23
Total shells . . . . . =		4790.0	99.97

These tabulations show that the two outer  $1\frac{3}{4}$  inch shells comprise over 78 per cent of the volume of the entire body.

#### 30 INCH TUBULAR TRIER (WITH BLOCK AT CENTER REMOVED).

The tubular trier is practically 30 inches long, is made from  $\frac{1}{2}$  inch brass tubing, and removes a straight-sided core, practically 24 inches long and  $\frac{5}{8}$  inch deep. The core for approximately 1 inch at each end is tapered gradually from the bottom upward. The distance from the lower end of the core to the point of trier is  $1\frac{5}{16}$  inches, and that from the upper end of the core to the base of the handle of the trier is  $4\frac{3}{8}$  inches.

The volume of the core was calculated and checked by experiment, in which the actual quantity removed by the trier was weighed and the weight obtained was converted to cubic inches. This volume was found to be 1.941 cubic inches. The volume at the tapered inch of the core at each end of the trier was found to be 0.06 cubic inches.

Area of section of core (if it were a complete circle) . . . . . = 0.129 sq. in.  
 Area of segment sliced off from this section . . . . . = 0.047 sq. in.

Area of section of true core . . . . . = 0.082 sq. in.

Area of segment =  $\frac{\text{rise of segment}}{\text{diameter}}$ . Corresponding area (.251 sq. in.)  $\times$  square of diameter.

Rise of segment =  $\frac{5}{16}$  inch; diameter =  $\frac{7}{8}$  inch.

The volume of any uniform segment of the trier = area of section of true core  $\times$  length.

Figure 1 shows the trier inserted diagonally in a body equivalent to a 140 pound sack of flour. The volume in cubic inches and percentage volume of the entire core for each segment of the core as determined by the position of the trier with respect to the various zones follow:

SEGMENT	LENGTH OF CORE inches	VOLUME inches	VOLUME OF ENTIRE CORE per cent
1 (lower end)	1.50	0.101	5.20
2	3.25	0.266	13.70
3	3.25	0.266	13.70
4	3.25	0.266	13.70
5	4.30	0.352	18.14
6	3.25	0.266	13.70
7	3.25	0.266	13.70
8 (upper end)	2.20	0.158	8.14
Total core =	24.25	1.941	99.95



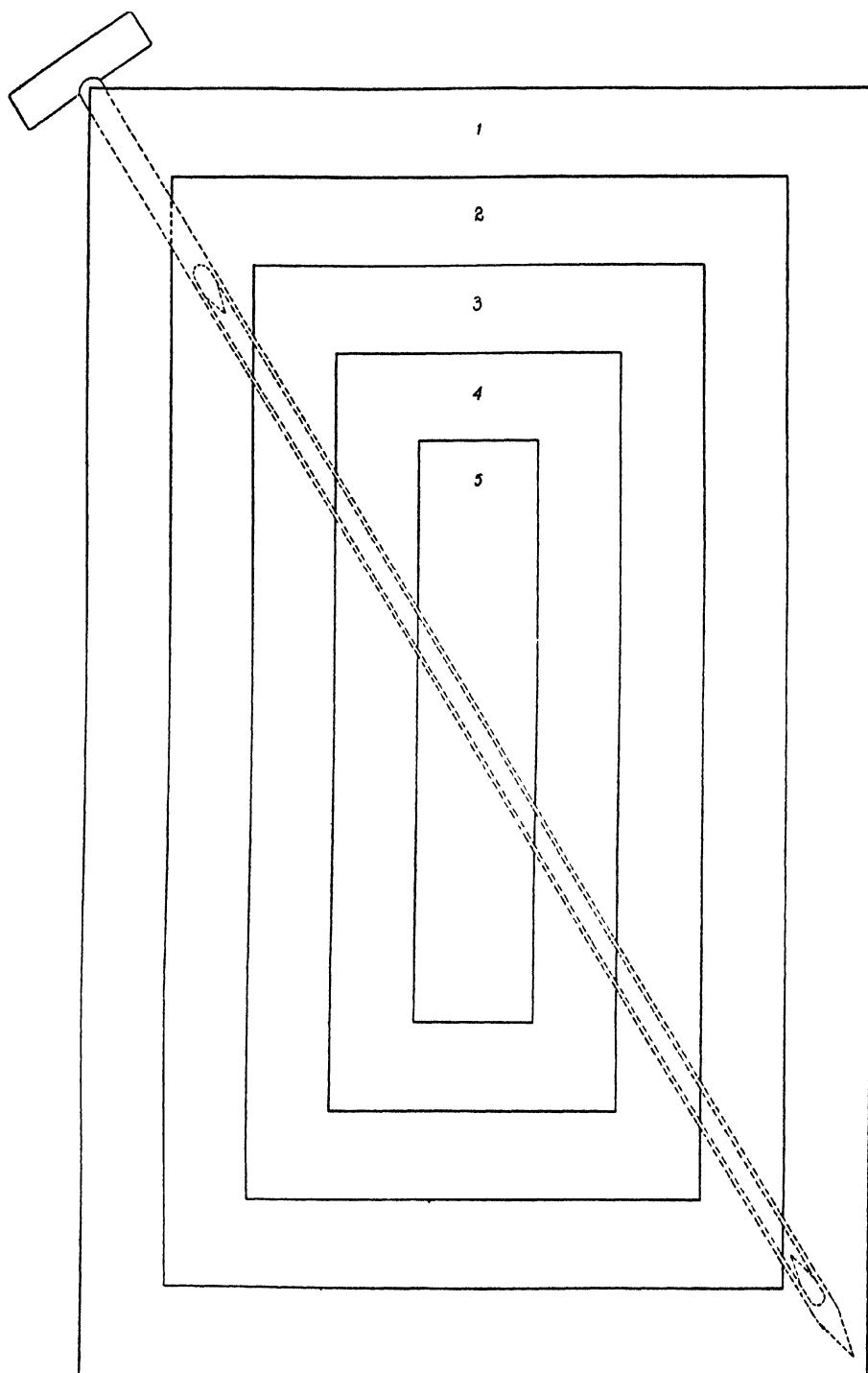


FIG. 1.—30 INCH TUBULAR TRIER INSERTED DIAGONALLY IN BODY EQUIVALENT TO A 140 POUND SACK OF FLOUR.

Carrying these calculations a step further, it is found that the trier removes the following quantities of material from the four  $1\frac{3}{4}$  inch zones and center:

ZONE	VOLUME OF CORE cu. in.	VOLUME OF ENTIRE CORE per cent	VOLUME THAT SHOULD BE TAKEN per cent
1	0.101	5.20	48.45
2	0.424	21.84	29.95
3	0.532	27.40	15.69
4	0.532	27.40	5.65
5 (center)	0.352	18.14	0.23
Total core =	1.941	99.98	99.97

Thus the 30 inch tubular trier removes a core comprising far too great a proportion of the three inner zones and too small a proportion of the two outer zones.

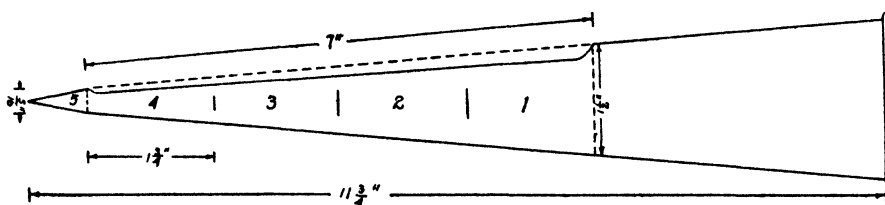


FIG. 2.—PROPOSED CONICAL TRIER, WHICH THEORETICALLY REMOVES A REPRESENTATIVE AND PROPORTIONATE CORE.

#### PROPOSED CONICAL TRIER (FIGURE 2).

The size of a conical trier, which theoretically removes a representative and proportionate core from the seven 1 inch (or four  $1\frac{3}{4}$  inch) zones of the body equivalent to a 140 pound sack of flour was calculated. The proposed trier and others that follow are considered to enter midway in the upper seam and to extend toward the center of the bag. Such a trier has a small base,  $1\frac{3}{8}$  inch in diameter, and a large base,  $1\frac{1}{2}$  inches in diameter, and draws a core 7 inches in length and a little over  $3\frac{3}{4}$  cubic inches in volume. The face of the cone is cut away by a plane to a depth of  $\frac{1}{16}$  inch at the small end and to a depth of  $\frac{3}{16}$  inch at the large end. The following tabulation shows the volumes of the seven 1 inch segments of the core:

SEGMENT	VOLUME cu. in.	VOLUME OF ENTIRE CORE per cent	VOLUME THAT SHOULD BE REMOVED per cent
1 (large end)	1.166	30.13	30.18
2	0.901	23.29	23.50
3	0.689	17.81	17.72
4	0.488	12.61	12.71
5	0.322	8.32	8.41
6	0.201	5.19	4.96
7 (small end)	0.101	2.61	2.24
Total core =	3.868	99.96	99.72

Based on calculations, this trier removes a practically representative core, as shown by checking the volume of each of the seven 1 inch segments of the core with the percentage volumes of the corresponding zones of the sack. It is at once apparent, however, that this trier is not practicable, owing to the fact that its insertion would make too large a hole in the sack of flour, the core at the larger end being  $1\frac{1}{2}$  inches in diameter.

Further consideration of proposed triers suggests the possibility of using a conical trier and a tubular trier with one tapered end, these triers to be of smaller bore and each to be inserted a number of times at varying depths.

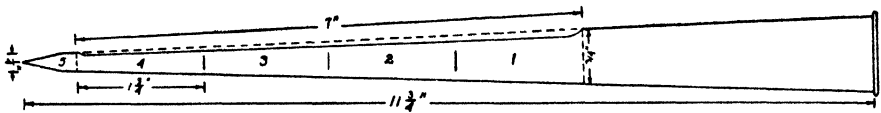


FIG. 3.—PROPOSED CONICAL TRIER TO BE INSERTED TWICE, THE FIRST TIME TO A DEPTH OF  $1\frac{1}{4}$  INCHES AND THE SECOND TO A DEPTH OF 7 INCHES.

#### PROPOSED CONICAL TRIER (FIGURE 3).

With one complete insertion this trier would remove a core 7 inches long with one base  $\frac{1}{4}$  inch in diameter and the other  $\frac{3}{4}$  inch in diameter, its volume being practically  $1\frac{1}{2}$  cubic inches. Calculation showed that such a trier would remove the most representative core if inserted twice, the first time to a depth of  $1\frac{1}{4}$  inches and the second to a depth of 7 inches. The volume of the core thus drawn would be practically  $1\frac{1}{2}$  cubic inches. The face of the cone is cut away by a plane to a depth of  $\frac{1}{2}$  inch and  $\frac{3}{4}$  inch at the small and large ends.

SEGMENT		VOLUME cu. in.	VOLUME OF ENTIRE CORE per cent	
	1 (large end)	0.597	43.89	
	2	0.400	29.41	
	3	0.241	17.72	
	4 (small end)	0.122	8.97	
Total core =		1.360	99.99	
SEGMENT	FIRST INSERTION $1\frac{1}{4}$ INCHES, REMOVED cu. in.	SECOND INSERTION 7 INCHES, REMOVED cu. in.	TOTAL REMOVED cu. in.	VOLUME OF CORES REMOVED per cent
1	0.122	0.597	0.719	48.51
2	None	0.400	0.400	26.99
3	None	0.241	0.241	16.26
4	None	0.122	0.122	8.23
Total core =			1.482	99.99
	1ST SEGMENT	2D SEGMENT	3D SEGMENT	4TH SEGMENT
Percentage that should be removed	48.45	29.95	15.69	5.65
Percentage removed by trier	48.51	26.99	16.26	8.23
Error =	+0.06	-2.96	+0.57	+2.58
Total error =	6.17 per cent.			

This trier, used as indicated, is accurate, removing a core representative with respect to the first segment, practically so for the third segment, about 3 per cent too small for the second segment, and about 2½ per cent too great for the fourth segment.

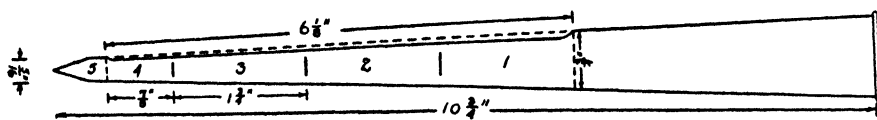


FIG. 4.—PROPOSED CONICAL TRIER TO BE INSERTED TWICE, THE FIRST TIME TO A DEPTH OF ½ INCH AND THE SECOND TO A DEPTH OF 6½ INCHES.

#### PROPOSED CONICAL TRIER (FIGURE 4).

This trier is similar to the trier shown in Figure 3, with the exception that the length of the core is 6½ inches and the diameter of the small base is ⅛ inch. With one complete insertion the volume of the core is practically 1⅛ cubic inches. The trier is to be inserted to a depth of ½ inch the first time and to a depth of 6½ inches the second time. The volume of the entire core thus drawn is approximately 1½ cubic inches.

SEGMENT	VOLUME cu. in.	VOLUME OF ENTIRE CORE per cent
1 (large end)	0.597	45 53
2	0.400	30 51
3	0.241	18 38
4 (small end)	0.073	5 56
Total core =	1 311	99 98

SEGMENT	FIRST INSERTION ½ INCH, REMOVED cu. in.	SECOND INSERTION 6½ INCHES, REMOVED cu. in.	TOTAL REMOVED cu. in.	VOLUME OF CORES REMOVED per cent
1	0.073	0.597	0.670	48 40
2	None	0.400	0.400	28 90
3	None	0.241	0.241	17 41
4	None	0.073	0.073	5 27
Total core =			1 384	99.98

	1ST SEGMENT	2D SEGMENT	3D SEGMENT	4TH SEGMENT
Percentage that should be removed	48.45	29 95	15.69	5 65
Percentage removed by trier	48 40	28 90	17 41	5 27
Error	-0 05	-1 05	+1 72	-0.38
Total error =	3.20 per cent.			

This trier, used as indicated, removes a core practically representative for segments 1 and 4, about 1 per cent too small for the second segment, and about 1½ per cent too great for the third segment, the total error being only a little over 3 per cent.

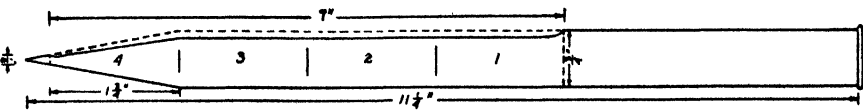


FIG. 5.—PROPOSED TUBULAR TRIER TO BE INSERTED TWICE, THE FIRST TIME TO A DEPTH OF 3 1/2 INCHES AND THE SECOND TO A DEPTH OF 7 INCHES.

PROPOSED TUBULAR TRIER (FIGURE 5).

With one complete insertion this trier would remove a core 7 inches long having a volume of practically 2 1/2 cubic inches. Segments 1, 2, and 3 are tubular and segment 4 is conical. This trier has bases for the tubular section 3/4 inch in diameter, while the diameter of the base at the small end is 1/8 inch. For the removal of the most representative core the trier should be inserted to a depth of 3 1/2 inches the first time, and to a depth of 7 inches the second time. The volume of the core thus removed would be practically 3 1/2 cubic inches. The face of the trier is cut away by a plane to a depth of 1/2 inch at the small end and to a depth of 3/8 inch on the tubular portion.

		SEGMENT	VOLUME cu. in.	VOLUME OF ENTIRE CORE per cent
		1 (outer end)	0.720	29.48
		2	0.718	29.40
		3	0.718	29.40
		4 (inner end)	0.286	11.71
		Total core =	2.442	99.99

SEGMENT	FIRST INSERTION 3 1/2 INCHES, REMOVED cu. in.	SECOND INSERTION 7 INCHES, REMOVED cu. in.	TOTAL REMOVED cu. in.	VOLUME OF CORES REMOVED per cent
1	0.718	0.720	1.438	41.72
2	0.286	0.718	1.004	29.13
3	None	0.718	0.718	20.83
4	None	0.286	0.286	8.32
Total core =			3.446	100.00

	1ST SEGMENT	2D SEGMENT	3D SEGMENT	4TH SEGMENT
Percentage that should be removed	48.45	29.95	15.69	5.65
Percentage removed by trier	41.72	29.13	20.83	8.32
Error =	-6.73	-0.82	+5.14	+2.67
Total error =	15.36 per cent.			

This trier removes a core containing practically the desired proportion of segment 2, but it is more than 6 1/2 per cent too low for the first segment and more than 5 per cent and 2 1/2 per cent too high for the third and fourth segments.

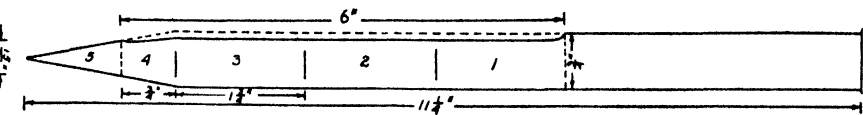


FIG. 6.—PROPOSED TUBULAR TRIER TO BE INSERTED TWICE, THE FIRST TIME TO A DEPTH OF 2 1/2 INCHES AND THE SECOND TO A DEPTH OF 6 INCHES.

A correctly designed trier will remove proportionate quantities of flour from the various zones, the amount to be taken from each zone being determined by the percentage volume of the entire sack represented in each zone.

A little over 71 per cent of the entire volume of a 140 pound sack of flour is contained in the three outer 1 inch zones; over 78 per cent of the entire volume is contained in the two outer  $1\frac{1}{4}$  inch zones. A 140 pound sack can be divided into seven 1 inch or four  $1\frac{1}{4}$  inch zones. The central portion remaining, being only 0.23 of 1 per cent of the entire cubical contents of the sack, can be disregarded.

The ratio of the percentage volumes of the four  $1\frac{1}{4}$  inch zones of a 140 pound sack of flour is practically 1 : 2 :  $3\frac{1}{4}$  : 5 for zones 4, 3, 2, and 1, No. 4 being the inner zone which is considered as unity. It follows that this ratio also applies to the corresponding segments of the desired core to be removed.

The Jabez Burns & Sons No. 4 trier removes too high a proportion of core from the inner and too low a proportion from the outer zones. The 30 inch tubular trier is open to the same criticism, but to a greater degree.

A conical trier, with small base  $\frac{1}{8}$  inch in diameter and large base  $1\frac{1}{2}$  inches in diameter, inserted to a depth of 7 inches (entire length of core), would remove a representative core  $3\frac{3}{4}$  inches in volume. Such a trier is not practicable, however, owing to the fact that its insertion would make too large a hole in the sack.

A conical trier, with a small base ( $\frac{1}{4}$  inch in diameter) and a large base ( $\frac{3}{4}$  inch in diameter), inserted twice, the first time to a depth of  $1\frac{3}{4}$  inches and the second time to a depth of 7 inches (length of one complete core), would remove a core which would be closely representative (the total error being a little over 6 per cent) and  $1\frac{1}{2}$  cubic inches in volume. The principal objection to this trier is the relatively small sample obtained by the two operations.

A conical trier having a small base ( $1\frac{5}{8}$  inch in diameter) and large base ( $\frac{3}{4}$  inch in diameter), inserted twice, the first time to a depth of  $\frac{7}{8}$  inch and the second to a depth of  $6\frac{1}{2}$  inches (length of one complete core), would remove a core remarkably representative (the total error being a little over 3 per cent) and  $1\frac{1}{2}$  cubic inches in volume. The chief objection to this trier also is the relatively small sample obtained in drawing a representative core.

A combined tubular and conical trier with small and large bases,  $\frac{1}{8}$  inch and  $\frac{3}{4}$  inch in diameter, inserted twice, the first time to a depth of  $3\frac{1}{2}$  inches and the second to a depth of 7 inches (the length of one complete core), yields a core showing an error of approximately  $15\frac{1}{2}$  per cent and having a volume of  $3\frac{1}{2}$  cubic inches. Of this total error  $11\frac{1}{2}$  per cent occurs in the first and third segments, corresponding to the first and third zones of the sack.

A combined tubular and conical trier with small and large bases  $\frac{1}{4}$  inch and  $\frac{3}{4}$  inch in diameter, inserted twice, the first time to a depth of  $2\frac{1}{2}$  inches and the second time to a depth of 6 inches (the length of one complete core), produces a core showing an error of approximately  $13\frac{1}{2}$

per cent and having a volume of  $3\frac{1}{8}$  cubic inches. Of this total error 11 per cent occurs in the first and third segments, corresponding to the first and third zones of the sack. This trier also shows a variation from the theoretical but has the advantage of yielding a comparatively large sample with each aggregate core.

In the second type of the conical trier (Figure 4) and in the combined conical and tubular trier (Figure 6) the fourth segment would not take a representative core from the corresponding zone or zones, owing to the fact that this segment as shown is not  $1\frac{1}{4}$  inches long, being  $\frac{7}{8}$  inch in the first and  $\frac{3}{4}$  inch in the second trier. For all practical purposes, however, this error is negligible.

### CONCLUSIONS.

The five types of proposed triers here considered seem to yield theoretical cores which are reasonably representative. The selection of any one as the most desirable will depend upon the relative importance of the various factors, such as volume and accuracy of core, size of trier, ease of operation, and cost of trier. The details of design of the triers shown should not be considered as final. For example, if it is expedient to collect smaller samples of flour than has heretofore been the practice, it might be advisable to increase the size of the opening in the triers and to equip each trier with a revolving shell to insure the drawing of an absolutely representative core. Or again, it might be advisable to slightly straighten the sides of the triers, making them U-shaped in cross section, thereby permitting greater ease and rapidity in filling and emptying the triers.

The designs as sketched are entirely adequate, however, for considering the comparative theoretical volume and accuracy of cores withdrawn.

### EFFECT OF STORAGE ON THE COMPOSITION OF A NOODLE AND JUDGING THE DEGREE OF DECOMPOSITION OF THE LIPOIDS.

By RAYMOND HERTWIG (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

Many illustrations of the decrease of the lecithin or lipid phosphoric acid of noodles during storage are cited in chemical literature. This information is quite general among food analysts, who consider it the chief uncertain factor in estimating egg solids by a formula method involving the determination of this substance. The interpretation of the analysis of an unknown noodle sample, therefore, is always open to question, since no means has been proposed for determining the extent to which this lipid phosphoric acid decomposition has progressed.



The only published data showing the change in composition of noodles during storage by the present method of the Association of Official Agricultural Chemists for the examination of noodles<sup>1</sup> are given by Buchanan in a preceding number of *This Journal*<sup>2</sup>. However, the

TABLE 1.  
*Results showing composition of noodle during storage.*

Analyst	R. Hertwig	J. C. Palmer*	L. H. Bailey†
Date of analyses	January, 1923	August, 1923	October, 1924
Moisture, per cent	12.26 12.36	.....	11.40 11.43
Lipoids, per cent	4.52 4.56 4.57	.....	4.07 4.12 4.14
Lipoid P <sub>2</sub> O <sub>5</sub> , per cent	0.079 0.080 0.081	.....	0.065 0.065 0.070
Fat (acid hydrolysis method), per cent	4.37 4.40	.....	4.37 4.38 4.28
Water-soluble protein-nitrogen precipitable by 40 per cent alcohol, per cent	0.155 0.157	0.153 0.156	0.146 0.151
1. $\frac{\text{Lipoid P}_2\text{O}_5 \times 100}{\text{Alcohol-precipitable nitrogen}}$	51.3	... .	45.3
2. $\frac{\text{Alcohol-precipitable nitrogen} \times 100}{\text{Lipoids}}$	3.4	.....	3.6
3. $\frac{\text{Alcohol-precipitable nitrogen} \times 100}{\text{Fat (acid hydrolysis method)}}$	3.6	.....	3.4
4. $\frac{\text{Lipoids}}{\text{Fat (acid hydrolysis method)}}$	1.04	... .	0.95
Calculated egg solids (moisture-free basis), per cent	3.4	.....	2.2

\* Food and Drug Inspection Station, San Francisco, Calif.

† Bureau of Chemistry, Washington, D. C.

analyses given do not include determinations for fat by the acid hydrolysis method. A marked decrease of lipid phosphoric acid was found after storage of the samples for one year and a very slight decrease of the water-soluble protein-nitrogen precipitable by 40 per cent alcohol.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 232.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 407.

The results given in Table 1 represent the composition of a noodle at different periods, approximately 22 months apart, by all the association methods for noodle analysis. The first analysis was made within one month after manufacture of the noodle. The ground sample was kept in a pint jar with a screw top having a cork lining and under ordinary laboratory conditions.

#### DEDUCTIONS.

The following deductions can be drawn from the results given in Table 1:

1. The composition of the solids of a noodle held under fairly dry conditions, similar to those of this experiment, during which no noticeable insect or mould development occurs, changes somewhat with respect to the lipoids but remains practically constant in so far as results by the other methods indicate.

2. The methods for fat (acid hydrolysis method) and water-soluble protein-nitrogen precipitable by 40 per cent alcohol determine those component substances that remain practically unaltered during the average conditions of storage of noodles and are therefore of especial importance for the proper interpretation of the analysis of an unknown sample.

3. The ratios (1), (2), and (3) are not altered sufficiently during storage of a noodle to interfere with their intended purpose of differentiating between whole egg and commercial yolk noodles. Ratios of the "alcohol-precipitable nitrogen" to lipoids and to fat (acid hydrolysis method), respectively, are approximately the same for noodles so long as no real serious decomposition of the lipoids occurs.

4. The ratio of lipoids to fat (acid hydrolysis method) indicates the degree of decomposition of the lipoids and lipid  $P_2O_5$ . This ratio is above unity in a fresh noodle before the lipoids have undergone much decomposition, but it approaches unity and then becomes less than unity as the lipoids gradually diminish during the storage of the noodle.

5. An urgent need exists for a method for estimating the egg solids content of a noodle by means of a component substance of noodles not susceptible to change under ordinary conditions of storage.

Ratios of lipoids to fat (acid hydrolysis method) taken from some available analyses of eggs, noodles, a flour, and a semolina are given in Table 2.

Although the results shown in Table 2 are limited, it is quite likely that average values for the ratio of lipoids to fat (acid hydrolysis method) for eggs and flours would fall approximately between 1.1 and 1.2. Consequently, this ratio should approximate at least 1.1 for those noodles in which the lipoids have undergone little or no decomposition. It is apparent, therefore, that this ratio for an unknown noodle sample indicates

TABLE 2.  
*Ratios of lipoids to fat.*

ANALYST	SAMPLE	LIPOIDS	FAT (ACID HYDROLYSIS METHOD)	LIPOIDS FAT (ACID HYDROLY- SIS METHOD)
		<i>per cent</i>	<i>per cent</i>	
R. Hertwig	Semolina	2.48 2.56	2.06 2.09	1.22
R. Hertwig	Flour	2.07	1.86	1.11
J. C. Palmer	Dried egg	50.04 50.04 50.02 49.90	45.88 45.98 46.04	1.09
L. H. Bailey	Fresh whole egg	12.79 12.87	11.00 11.09	1.16
R. Hertwig	Fresh whole eggs	13.69 13.69	12.12 12.23	1.12
R. Hertwig	Dried whole eggs	46.70	41.53	1.12
R. Hertwig	Noodle of known com- position (fresh)	4.42 4.49	3.86 3.89	1.15
R. Hertwig	Noodle of known com- position (1 year old)	4.13	3.77	1.09
R. Hertwig	Commercial noodle	4.39 4.40	4.27 4.31	1.02
R. T. Elliott*	Commercial noodle	4.41 4.46	4.33 4.47	1.01
R. T. Elliott	Commercial noodle	3.22 3.32	2.80 2.85 2.88	1.15
R. T. Elliott	Commercial noodle	3.92 3.98	3.25 3.34	1.20
T. O. Kellems†	Commercial noodle	4.13 4.13 4.16	4.27 4.35	0.96
T. O. Kellems	Commercial noodle	4.13 4.26	3.87 3.89	1.08

\* Food and Drug Inspection Station, Seattle, Wash.

† Food and Drug Inspection Station, San Francisco, Calif.

the extent to which decomposition of the lipoids has occurred. It also indicates the degree of dependability of the estimated egg solids by the formula method involving the lipid phosphoric acid.

Investigation may disclose some means of arriving at the original lipid  $P_2O_5$  content of a noodle before any decomposition has occurred in the lipoids by calculations based on the lipoids to fat (acid hydrolysis

method) ratio. A factor for each ratio value likely to occur in a noodle may be evolved mathematically for multiplying the determined lipid  $P_2O_5$  in an unknown sample so that the resultant value applied in the formula for calculating the egg solids will give the approximate true egg solids content of the noodle.

## MODIFIED KERR-SORBER METHOD FOR UNSAPONIFIABLE MATTER IN FATS AND GREASE.

By RAYMOND HERTWIG, G. S. JAMIESON, W. F. BAUGHMAN, and L. H. BAILEY (Bureau of Chemistry, Washington, D. C.).

The Association of Official Agricultural Chemists, during 1924, studied the determination of unsaponifiable matter in fats and grease by three methods: the Kerr-Sorber method<sup>1</sup>, a modified A. O. A. C. method<sup>2</sup>, and the method of the Committee on Analysis of Commercial Fats and Oils of the American Chemical Society<sup>3</sup>. Several collaborators found considerable quantities of free fatty acids or acid soaps in the residues obtained by the Kerr-Sorber method. On the other hand, only small quantities of these acid substances were found in the residues from the Committee method by one of the collaborators. Since then, these observations have been confirmed by the authors. The titratable acidity of the residues by the Kerr-Sorber method was found to range from 0.5–1.1 ml. of 0.1 *N* alkali as compared to 0.05–0.15 ml. by the Committee method. It is evident, therefore, that considerable hydrolysis of the soap takes place in the Kerr-Sorber method during the washing of the ether with the large volumes of water.

The chemical literature discloses a previous method for the determination of unsaponifiable matter that provides against the extraction of fatty acids along with the unsaponifiable matter. Thaysen<sup>4</sup>, in a study of the determination of cholesterin and cholesterin esters, proposed a method quite similar to that of Kerr and Sorber for the extraction of these substances from lipoids. In this method the soap is washed out of the ether solution of the unsaponifiable matter with dilute alkali. This procedure tends to avoid hydrolysis of the soaps and the consequent extraction by the ether of the derived fatty acids. The concentration of the alkaline wash solution is not specified by Thaysen. Fex<sup>5</sup> successfully employs this particular procedure of Thaysen for the extraction of unsaponifiable matter from body organs.

Many experiments were made in attempting to overcome the hydrolysis of the soap and the subsequent extraction of free fatty acids in the

<sup>1</sup> The Cotton Oil Press, 1924, 7: 40; *J. Assoc. Official Agr. Chemists*, 1924, 8: 90.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, unpublished.

<sup>3</sup> *Ibid.*, 1924, 8: 85.

<sup>4</sup> *Biochem. Z.*, 1914, 62: 89.

<sup>5</sup> *Ibid.*, 1920, 104: 82.

*Unsaponifiable matter.*

ANALYSTS	MODIFIED KERR-SORBER METHOD	COMMITTEE METHOD		
		5 Extractions	7 Extractions	9 Extractions
SAMPLE A				
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
G. S. Jamieson	0.20	0.18	0.20	....
	0.20		0.22	
W. F. Baughman	0.20	....	....	....
	0.20			
L. H. Bailey	0.17	....	....	....
	0.18			
SAMPLE B				
G. S. Jamieson	0.55	0.55	0.60	....
	0.56	0.57	0.61	
W. F. Baughman	0.54	....	....	....
	0.58			
L. H. Bailey	0.56	....	....	....
	0.53			
SAMPLE C				
G. S. Jamieson	1.32	0.97	1.13	....
	1.34	0.99	1.19	
W. F. Baughman	1.34	0.98	1.14	1.34
	1.29		1.15	1.40
L. H. Bailey	1.22	....	...	....
	1.26			
Dorothy Paine Bureau of Chemistry Washington, D. C.	1.21	....	....	....
	1.28			
	1.29			
	1.32			
SAMPLE D				
G. S. Jamieson	1.89	1.79	1.91	....
	1.93	1.80	1.93	
	1.96			
W. F. Baughman	1.98	1.76	1.93	....
	2.00	1.82	2.00	
L. H. Bailey	1.92	....	....	....
	2.02			
Dorothy Paine	1.94	....	....	....
	1.98			
	2.03			
Raymond Hertwig	1.97	....	....	....
	2.00			
	2.02			

case of the Kerr-Sorber method. The ether-soap solution was washed first with three successive 100 ml. portions of dilute potassium hydroxide solution ranging in concentrations from 0.1–1.0 normality. The ether then was washed with successive 30 ml. portions of water to remove the alkali. The concentration that appeared to be the most suitable for the purpose was a 0.2 *N* potassium hydroxide solution. Results on four samples of oils and greases analyzed in this manner are shown in the table. The titratable acidity obtained in these instances was found to be negligible. Some comparative results by the Committee method also are given.

The results indicate that a slight modification of the Kerr-Sorber method, involving the washing of the soap out of the ether solution of the unsaponifiable matter with 0.2 *N* potassium hydroxide solution, practically removes the only known objection raised to this splendid method, namely, the extraction of a small quantity of fatty acids with the unsaponifiable matter. A description of this modified method follows:

#### *Modified Kerr-Sorber Method.*

##### REAGENTS AND APPARATUS.

(a) *Concentrated potassium hydroxide solution.*—100 grams of potassium hydroxide dissolved in 100 ml. of water.

(b) *Dilute potassium hydroxide solution, approximately 0.2 N.*—11.2 grams of potassium hydroxide dissolved in 1000 ml. of water.

(c) *Ethyl alcohol.*—Approximately 95 per cent by volume.

(d) *Ethyl ether.*—U. S. P.

(e) *Phenolphthalein solution.*—1 gram of phenolphthalein dissolved in 100 ml. of alcohol.

##### APPARATUS.

(a) *Separatory funnel.*—500 ml. capacity, ether-tight. The glass connections are lubricated with water.

(b) *Erlenmeyer flask or beaker-flask for saponification.*—100–200 ml. capacity.

(c) *Erlenmeyer flask or beaker-flask.*—250 ml. capacity.

##### PROCEDURE.

Accurately weigh about 5 grams of sample into the saponification flask. Add 30 ml. of the alcohol and 3 ml. of the concentrated potassium hydroxide solution. Place a small, short-stemmed funnel in the neck of the flask to serve as a condenser. Boil gently on the steam bath for about 20 minutes or until complete saponification occurs. Cool to about 30°C., add 50 ml. of ether, mix, and transfer to the separatory funnel. Rinse the flask with two successive 50 ml. portions of ether, add to the separatory funnel, and mix thoroughly. Wash the saponification flask with 100 ml. of the dilute potassium hydroxide solution and pour into the separatory funnel in a slow, steady stream. Rotate the funnel very gently to secure better contact of the solutions but do not shake. (Shaking at this stage brings about stubborn emulsions.) Allow the liquids to separate completely and then slowly draw off as much of the soap solution as possible. Do not draw off any layer of emulsion that may be formed. Keep the volume of the ether at about 150 ml. by replacing that dissolved by the wash solutions. Further treat the

ether solution with two successive 100 ml. portions of the alkaline wash solution in the manner described previously. Add 30 ml. of water to the ether and rapidly rotate the liquid layers. When the layers have separated completely, draw off the water. Repeat this treatment until the washings are free from alkali, as shown by testing with phenolphthalein. Three washings usually suffice. Transfer the ether solution quantitatively through a pledget of cotton in the stem of a funnel to the weighed 250 ml. Erlenmeyer flask or beaker-flask. Before weighing the flask dry it in an oven at 100°C., and then allow it to stand in the air to constant weight. Distil off the ether and dry the flask and residue at 100°C. until no further loss in weight occurs. Allow the flask with unsaponifiable matter to come to equilibrium with the atmosphere before weighing. Deduct from the weight of the unsaponifiable matter any blank obtained from the reagents used.

## A TEST OF ASCARITE, A CARBON DIOXIDE ABSORBENT, AS ITS OWN DRIER.

By FRANKLIN W. MARSH (Office of Soil Bacteriology, Bureau of Plant Industry, Department of Agriculture).

In preliminary experiments on the determination of the quantities of carbon dioxide evolved from soils, an effort was made to adapt to this work the gravimetric method described by Stetser and Norton<sup>1</sup>, in which a new absorbent, Ascarite, was used. This material is a special mixture of sodium hydroxide and asbestos that has been found by its originators to give excellent results when used in the analysis of steels for carbon.

When working with soils the gases must be drawn or forced through the absorbing medium for periods varying from 12–48 hours or more for each determination instead of the 5 or 10 minutes ordinarily used in steel analysis, so that necessarily conditions are widely different in these two cases. It was hoped, however, that a means of adaptation of the method might be found.

### EXPERIMENTAL.

Midvale absorption bulbs (Stetser and Norton modification) were filled with Ascarite, as directed by Stetser and Norton, and placed in a simple train in which air, previously freed from carbon dioxide, was dried and then run through the bulbs. Both suction and pressure were tried for propelling the air through the train, and as it was found that pressure gave slightly better results it was used in all the tests from which the data given in this paper were taken. The first experiments showed a marked loss of weight from the absorbent, and it was found, by placing a Midvale bulb filled with phosphoric anhydride at the outlet of the Ascarite-filled bulb and weighing it before and after each of several runs, that the reduction in weight was due to loss of moisture from the Ascarite. Four freshly filled bulbs were then carefully tested for periods of 2, 5,

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<sup>1</sup> *Iron Age*, August 22, 1918.

18, and 48 hours, and the results obtained are given in the table. A check bulb was maintained under the same conditions as the others except that no gases were run through it. The changes of weight of this bulb will also be found in the table.

*Results showing milligrams of weight lost in various periods.*

BULB NO.	2 HOURS	5 HOURS	18 HOURS	48 HOURS
6	+ 0.10	- 0.70	- 1.30	- 7.70
7	+ 0.20	- 0.50	- 1.50	- 8.20
3	+ 0.05	- 0.60	- 3.80	- 16.30
4	+ 0.20	- 0.30	- 2.30	- 13.30
6	± 0.00	± 0.00	- 3.50	- 20.00
7	± 0.00	± 0.00	- 1.80	- 14.30
3	- 0.25	- 0.55	- 3.20	- 26.30
4	- 0.40	- 1.00	- 2.90	- 24.00
6	+ 0.20	+ 1.80*	- 1.70	- 31.50
7	+ 0.05	- 0.90	- 1.70	- 19.70
3	+ 0.10	- 2.20	- 1.50	- 35.40
4	± 0.00	- 1.10	- 1.20	- 38.00
6	- 0.30*	+ 1.40*	- 6.40	+ 36.90*
7	- 0.90	- 1.20	- 4.50	- 4.80
3	- 1.80	- 1.90	- 9.00	- 20.80
4	- 1.20	- 1.60	- 9.30	- 19.80
Total	- 3.65	- 12.55	- 55.60	- 300.10
Average	- .24	- .90	- 3.47	- 20.01

CHECKS.

5	+ 0.3	± 0.00	+ 0.50	+ 0.50
5	- 0.1	- 0.10	- 0.80	+ 1.40
5	+ 0.15	- 0.60	+ 0.75	+ 1.80
5	+ 0.20	+ 0.10	- 0.10	- 0.50
Total	+ .55	- 0.60	+ .35	+ 3.20
Average	+ .14	- 0.15	+ .09	+ 0.80

\* As No. 6 showed a slight leak these results were not included in the averages.

DISCUSSION.

The tabulated results indicate that there was a loss of weight from the Ascarite, increasing with the length of time the train was run. Up to 5 hours the losses seem to be within the limits of error of the experiment, but beyond this point they exceed this limit, and in periods of 48 hours they reach an average of over 20 mg. of lost moisture for each bulb. The speed at which the air was passed through the absorbent had an effect on the extent of the losses observed without a doubt, but as no meter was available when these preliminary experiments were made an exactly uniform flow of gas could not be maintained. However, the



speed was kept low and as nearly uniform as possible, and such fluctuations as occurred obviously do not affect the conclusions drawn from the data.

#### SUMMARY.

The results of these experiments confirm the findings of Stetser and Norton that Ascarite requires no additional drier if the running time is relatively short. However, where long runs are necessary, as in the determination of carbon dioxide production from soil, Ascarite, because of loss of moisture, can not be successfully used.

### THE "NEUTRALIZING VALUE" OF MONOCALCIUM PHOSPHATE.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.).

Monocalcium phosphate is used extensively by manufacturers of baking powder and self-rising flour; in these products it serves to liberate carbon dioxide from sodium bicarbonate.

Sodium bicarbonate, as found in the trade, is exceptionally uniform in composition, but monocalcium phosphate is more variable. Therefore, in order to make baking powders and self-rising flours properly, it is necessary for the manufacturer to know the reacting proportions of the monocalcium phosphate in question and the sodium bicarbonate under the conditions of baking. The number of parts of sodium bicarbonate that react with 100 parts of monocalcium phosphate in aqueous solution at the boiling temperature is known as the "neutralizing value" of monocalcium phosphate in terms of sodium bicarbonate.

According to Wadman<sup>1</sup>, there are two general procedures in use for determining this "neutralizing value": (1) Titration with standard alkali; (2) determination of the quantity of carbon dioxide evolved from sodium bicarbonate by a known weight of the phosphate. Wadman<sup>1,2</sup> however, shows that the various methods produce different results, while Catlin<sup>3</sup> concludes that there is no definite relation between the neutralizing value as obtained by titration and that obtained by gas evolution.

Collaborative studies of titrametric methods for the determination of the neutralizing value of monocalcium phosphate have been made by the Association of Official Agricultural Chemists<sup>4</sup>. It was found that the end points with indicators in solutions of phosphates were indefinite and rendered such methods unsatisfactory. Also the use of an outside indicator, as suggested by Hartmann<sup>5</sup>, was found to give results that did not indicate true neutralizing values.

<sup>1</sup> *J. Am. Chem. Soc.*, 1894, 16: 333.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1921, 12: 1146.

<sup>3</sup> *J. Anal. Chem.*, 1890, 4 (part 4): 361.

<sup>4</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 514; 1923, 6: 445; 1924, 8: 91.

<sup>5</sup> *Ibid.*, 1920, 3: 410.

When moisture is added to a baking powder, the acid present reacts with the bicarbonate of soda, liberating carbon dioxide gas and leaving a "residue" that may be alkaline, neutral, or acid. The reaction will depend upon the relative proportion of the acid-reacting material to the bicarbonate of soda. It was observed that baking powders prepared according to the neutralizing values obtained by titrametric methods invariably gave residues that reacted alkaline toward indicators and yielded appreciable quantities of residual carbon dioxide. Mixtures of monocalcium phosphate and sodium bicarbonate in equivalent proportions, as implied by the term "neutralizing value", should be expected to yield residues having no residual carbon dioxide and reacting neutral indicators. Any residual carbon dioxide, as well as an alkaline residue, indicates an excess of bicarbonate over that necessary to react with the phosphate. Therefore, it appears that the titrametric methods, besides being unsatisfactory as methods, do not determine the true neutralizing value of monocalcium phosphate. On the other hand, they give values corresponding to mixtures yielding alkaline residues under the conditions of baking.

It is apparent that at the present time the "neutralizing value" of monocalcium phosphate varies with the method employed. There is need, therefore, for an accurate method that will be generally accepted. On this account, an attempt was made to find some method that would give as nearly as possible the actual quantity of sodium bicarbonate that would react with the phosphate under baking conditions. For this purpose, a hydrogen-ion concentration method appeared to offer possibilities.

The applicability of such a method was tried on twelve experimental baking powders. These powders were made from a fixed weight of sodium bicarbonate and such weights of commercial monocalcium phosphate as correspond to the ratios shown in Table 1.

The hydrogen-ion concentration of a solution of each of these baking powders was obtained. The solutions were prepared by boiling one gram of the sample with 100 cc. of distilled water until free from carbon dioxide. When cool, the hydrogen-ion concentrations of the supernatant liquids were obtained colorimetrically. In addition, the pH of four of the samples was determined electrometrically by R. M. Hann of the Bureau of Chemistry.

Residual carbon dioxide determinations also were made on each of these baking powders by the A. O. A. C. tentative gasometric method as described in *The Journal*<sup>1</sup>. These results are shown in Table 1 in connection with pH values obtained by the method described in this paper.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 454.

TABLE 1.  
Results obtained on residues of experimental baking powders.

SAMPLE NO.	SODIUM BICARBONATE	MONOCALCIUM PHOSPHATE	RATIO OF BICARBONATE TO PHOSPHATE (x : 100)	RESIDUAL CARBON DIOXIDE	H-ION CONCENTRATION	
					Colorimetric	Electrometric
	grams	grams	x	per cent	pH	pH
3a	26.73	32.60	82	0.68	9.0	....
4a	26.73	33.41	80	0.35	8.0	....
5a	26.73	34.27	78	0.35	7.7	....
6a	26.73	35.17	76	0.21	7.5	....
7a	26.73	36.12	74	0.17	7.4	....
8a	26.73	37.12	72	0.17	7.3	....
9a	26.73	38.19	70	0.12	7.3	....
10a	26.73	39.31	68	0.06	7.1+	7.18
11a	26.73	40.50	66	0.04	7.1-	7.08
12a	26.73	41.77	64*	none	7.0	7.01
13a	26.73	43.11	62	none	6.9	6.97
14a	26.73	44.55	60	none	6.8	....

\* Actual neutralizing value.

The results given in Table 1 show that Sample 12a contained the proper proportion of bicarbonate and phosphate to produce a neutral residue as indicated both by the hydrogen-ion concentration and by the residual carbon dioxide. This sample was also the first of the baking powders, arranged in the order of their proportionately decreasing bicarbonate contents, to yield no residual carbon dioxide. All the preceding samples showed some residual carbon dioxide and yielded alkaline solutions indicating excessive proportions of bicarbonate; while, on the other hand, the samples following 12a gave no residual carbon dioxide and yielded acid solutions indicating insufficient proportions of bicarbonate. Therefore, the correct neutralizing value of this sample of monocalcium phosphate is 64. The tentative A. O. A. C. titration method<sup>1</sup> gave a neutralizing value of 77 for this same phosphate.

The following method is offered for determining the true neutralizing value of monocalcium phosphate:

To 26.73 grams of sodium bicarbonate add 41.77 grams of the monocalcium phosphate to be tested (this assumes a neutralizing value of 64) and also add, if desired, 31.50 grams of neutral starch and mix thoroughly. Keep dry until used. Add one gram of this test baking powder to 100 cc. of distilled water in a 250 cc. Pyrex beaker. Boil until free from carbon dioxide. When cool, determine the pH of the supernatant liquid electrometrically or colorimetrically, using appropriate indicators.

26.73 grams of sodium bicarbonate, 41.77 grams of monocalcium phosphate, and 31.50 grams of starch, thoroughly blended, produce 100 grams of baking powder having a total carbon dioxide content of 14 per cent.

Should this trial indicate a pH other than 7.0, prepare other test baking powders either singly or in series, as above, using more or less of the phosphate as required.

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1925, 306.

The pH values should be checked by determining the residual carbon dioxide, and no residual carbon dioxide should be obtained with a pH of 7.0 or less.

The method presented is intended to determine the true neutralizing value of monocalcium phosphate and does not represent the actual proportions to be used in making baking powders. Usually commercial baking powders are intentionally made slightly alkaline and, therefore, properly contain greater proportions of sodium bicarbonate than those indicated by this method.

The pH values of some commercial baking powders obtained colorimetrically are shown in Table 2. The residual carbon dioxide values of these same powders were determined by J. I. Palmore of the Bureau of Chemistry.

TABLE 2.  
*Results obtained on residues of commercial baking powders.*

SAMPLE NO.	RESIDUAL CO <sub>2</sub>	pH (COLORIMETRIC)
	<i>per cent</i>	
1	2.38	9.8 +
2	1.23	9.1 +
3	1.10	9.2 -
4	0.55	8.3 +
5	0.53	8.7
6	0.48	8.5
7	0.43	8.4
8	0.42	8.4
9	0.38	8.2
10	0.33	8.4 -
11	0.23	7.5

Acknowledgment is hereby made of the helpful suggestions of R. Hertwig of the Bureau of Chemistry in the preparation of this paper.

## DETERMINATION OF THE SALT CONTENT OF CLAMS<sup>1</sup>.

By D. B. DILL (U. S. Food and Drug Inspection Station,  
San Francisco, Calif.).

The salt content of shellfish, particularly of fresh, shucked oysters, is often determined when "soaking" is suspected. The process of soaking involves imbibition by the freshly shucked meats of fresh water with associated leaching out of soluble electrolytes. The salinity of molluscan tissue is ordinarily calculated from the chlorine content of the ash, as for example in Smith's study of chemical changes in the oyster<sup>2</sup>. Recent

<sup>1</sup> From the U. S. Food and Drug Inspection Station, Seattle, Wash. This investigation was carried on under the direction of A. W. Hansen. Valuable criticisms were offered by C. L. Alsberg.

<sup>2</sup> U. S. Dept. Agr. Bull. 740.

unpublished observations by Bureau of Chemistry investigators indicate that this method of detecting soaking is not entirely reliable. It was the opinion of the writer that the unreliability of the method might be due in part to variable losses of chlorine occurring during ashing.

The changes that take place in the relative proportion of the mineral constituents of foods during ashing have been studied by Pfy<sup>1</sup>. He points out that the sulfur and phosphorus of organic compounds are oxidized to the corresponding acids, and if insufficient base is present, the more volatile acids, including hydrochloric, are displaced.

While the method of the Association of Official Agricultural Chemists<sup>2</sup> for determining chlorides in meat extract involves the addition of sodium carbonate solution before ashing, it is believed that this precaution is rarely observed in determining the chlorides (and indirectly the sodium chloride) in shellfish. An investigation of the loss of chlorine that may occur in ashing various species of shellfish, therefore, has been carried out.

The usual procedure for ashing was followed except that in one series 5 cc. of 5 *N* sodium carbonate solution was added to the 5 grams of sample. The samples were evaporated to dryness on the steam bath, charred, and ashed at low red heat. After weighing, the ash was dissolved in dilute nitric acid, chlorine was determined by the volumetric thiocyanate method, and sodium chloride was calculated therefrom.

Moisture was also determined, and the other data were calculated to the dry basis. In the series to which sodium carbonate had been added, the ash content was estimated by deduction of the weight of sodium carbonate added, as determined in a blank. The figures for the treated samples, therefore, merely represent an approximation of the ash content as determined on an untreated sample and are not intended to include a correction of the ash percentage for loss of chlorine. The results are presented in the accompanying table.

It is evident that the loss of chlorine that occurs in ashing shellfish without preliminary addition of sodium carbonate (or similar substance) is large and variable. It is suggested that a reinvestigation of the salt content method of detecting "soaking", observing the precaution of preventing the loss of chlorine in ashing might be profitable. It also appears that when ashing with sodium carbonate has been carried out a fair approximation of the ash content may be had by deducting the weight of added sodium carbonate.

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<sup>1</sup> *Z. Nahr. Genussm.*, 1922, 43: 313-39.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 250.

A NOTE ON THE INDOL CONTENT OF CANNED CRUSTACEA<sup>1</sup>.

By D. B. DILL and P. B. CLARK (U. S. Food and Drug Inspection Station, San Francisco, Calif.).

The relation between decomposition and the appearance of indol and skatol in canned salmon has been studied by Houghton and Hunter<sup>2</sup> and Clough<sup>3</sup>. They found that decomposition is usually, but not always, associated with the presence of indol or skatol, or both. They failed to find indol or skatol in normal freshly caught salmon either before or after canning. "The canning process," writes Clough, "does not appear to increase or decrease the amount of these compounds already present in partially spoiled salmon".

A number of commercial packs of crab meat, lobster meat, and shrimp meat have been examined in the Seattle and San Francisco laboratories of the Bureau of Chemistry. Ehrlich's test for indol, essentially as modified by Clough, was employed. These tests frequently have been found to be strongly positive.

On the basis of this finding it seemed worth while to determine whether or not indol (or some substance giving the same color reaction) is a normal constituent of crustacean flesh. Several pounds of Dungeness crab (*Cancer magister*), Puget Sound shrimp (species unknown), and of California spiny lobster (*Panulirus productus*) were obtained. These were dropped, while alive, into boiling dilute brine and boiled for 30 minutes after which they were removed, cooled, and shelled. This is a representative commercial method of preparing crustacea for canning. The meat was then washed with water, a representative sample was used for an indol test, and the remainder was packed in cans that were sealed and sterilized under 10 pounds steam pressure for 90 minutes. Examination of these samples for indol gave, in most cases, distinctly positive tests in the freshly cooked meat and in the meat immediately after canning as well as six months after canning.

A further experiment was carried out with live California spiny lobsters. Two of these were prepared exactly as before; two were vivisected. Examinations were made for indol with the following results:

Washed body meat from vivisected lobsters: Negative.

Viscera from vivisected lobsters: 20-25 mg. per 100 grams.

Body meat from cooked lobsters: 1.5 mg. per 100 grams.

Viscera from cooked lobsters: Positive<sup>4</sup>.

Water in which lobsters were cooked: Trace<sup>5</sup>.

<sup>1</sup> From the Seattle and San Francisco Food and Drug Inspection Stations, Bureau of Chemistry. Some of the indol determinations were made by D. H. McIntyre, to whom thanks are due. Especial indebtedness is also acknowledged to C. L. Alaberg for advice and suggestions.

<sup>2</sup> Pacific Fisherman, 1920, 18: 38-9.

<sup>3</sup> Publications Puget Sound Biol. Sta., 1922, 3: 195-272.

<sup>4</sup> A portion of this sample was lost. The test for indol on the remainder exceeded the standard of 1 mg.

<sup>5</sup> The color test on this sample was distinct, but less intense than the standard.

*Effect of sodium carbonate addition preliminary to ashing on the chlorine content of the ash of certain shellfish.*  
(Results calculated to the dry basis.)

NO.	SPECIES	NATURE OF SAMPLE	TREATED WITH Na <sub>2</sub> CO <sub>3</sub>		UNTREATED		NaCl† lost (PERCENTAGE OF TOTAL NaCl)
			"Ash"*	NaCl†	Ash	NaCl†	
1	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> )	Meat and nectar	per cent 22.30	per cent 16.30	per cent 21.50	per cent 13.80	15.3
2	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> )	Meat and nectar	24.69	15.83	24.06	13.86	12.4
3	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> )	Meat	10.83	4.75	9.88	2.74	42.3
4	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> )	Nectar	64.85	53.95	64.04	46.59	13.5
5	Soft-shell clam ( <i>Mya arenaria</i> )	Meat and nectar	24.04	17.16	....	12.75	25.7
6	Soft-shell clam ( <i>Mya arenaria</i> )	Meat and nectar	25.95	15.57	26.80	13.13	15.7
7	Butter clam ( <i>Saxidomus giganteus</i> )	Meat and nectar	15.78	9.10	15.71	6.84	24.8
8	Cockle ( <i>Cardium corbis</i> )	Meat and nectar	19.30	10.25	19.90	7.94	22.5

\* The figures in this column do not include a correction for the chlorine lost in the corresponding untreated sample.

† Calculated from the chlorine content. Sodium is of course not lost, but since the content of sodium chloride in foods is commonly calculated from the chlorine content, the results have been expressed as above.

It appears, therefore, that indol is not a normal constituent of the flesh of the spiny lobster. Its presence in canned crustacean meat may be due to its escape from the alimentary tract into the flesh during cooking, as in the case of the spiny lobster.

## RAPID ROUTINE METHOD FOR TOTAL SOLIDS DETERMINATION IN EGGS.

By RAYMOND HERTWIG and L. H. BAILEY (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

A vacuum method for determining total solids in eggs<sup>1</sup> was recommended for adoption as an "official method" at the 1924 meeting of the Association of Official Agricultural Chemists. The studies of the associate referees on liquid and dried eggs<sup>1</sup>, M. L. Hitchcock and J. C. Palmer, led to the recommendation of this method as an umpire method for this determination. The conditions defined by the method for drying, namely 98°–100°C. and a pressure equivalent to not more than 25 mm. of mercury, recommend themselves as practical and basic for removing the water from the sample and for giving precise and reproduceable results. Further, the method harmonizes with other methods now existent or in process of development for total solids in other food products, especially cereal and egg food preparations, and permits simple and readily intelligible comparison of results on a total solids basis obtained by the same method. This vacuum method, therefore, is clearly fitted for its intended purpose as a conventional umpire method for total solids in eggs.

The vacuum method, however, requires rather elaborate equipment and considerable time and therefore is not an economical method for general use. Consequently, this investigation was undertaken to develop a simple, rapid method for total solids determination that would give results approximating those of the umpire method and would qualify for adoption by the association as a routine method.

König<sup>2</sup>, Abderhalden<sup>3</sup>, and Lunge and Berl<sup>4</sup> propose the total solids determination of eggs by drying at from 100°–110°C. to approximately constant weight. The procedure and equipment necessary for drying egg samples at such temperatures and at atmospheric pressure appeared favorable for the development of a simple method. Accordingly, the behavior of egg, subjected to temperatures of from 110°–130°C. in an air oven for different periods of time, was investigated.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, unpublished.

<sup>2</sup> *Unter. Nahr. Genuss. und Gebrauchs.* III Band, 2. Teil, 4 Afl., p. 167.

<sup>3</sup> *Handbuch biolog. Arbeitsmethoden*, Abt. IV, Teil 8, Heft 2, 1922, p. 530.

<sup>4</sup> *Chem.-tech. Untersuchungsmethoden*, 6 Afl., Band 4, p. 346.



*Total solids in eggs.*

UMPIRE VACUUM METHOD, 98-99°C., 25 MM. MERCURY PRESSURE, 5 HOURS		AIR OVEN METHODS											
		per cent	TEMPERATURE	TIME OF DRYING									
				0.25 hour	0.5 hour	0.75 hour	1 hour	1.25 hours	1.6 hours	2 hours	18 hours	41 hours	
		°C.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
COMMERCIALLY DRIED EGG YOLK													
96.08*	96.69	110	..	....	....	96.16	....	....	96.10	....	95.33		
96.08						96.18			96.12	....	95.33		
96.09*	96.66	112	..	...	96.14	....	96.14	..	...	....	....		
96.12					96.18		96.15			....	....		
....	....	115	..	....	96.15	96.02	96.07	..	....	95.17* 95.41*	....		
					96.16	96.10	96.10			95.20 95.47	....		
....	....	119	..	....	96.04	..	95.95	....	....	....	....		
					96.05		95.96				....		
....	....	130	96.02	95.85	95.89	95.94	...	95.78	....	....	....		
			96.08	95.89	95.99	95.97		95.80	....	....	....		

\* Obtained on different days.

Total solids in eggs.—Concluded.

AIR OVEN METHODS											
VACUUM METHOD, 55°C., 13 CM. PRESSURE, 5 HOURS		TEMPERA- TURE °C.	TIME OF DRYING								
			1 hour	1.5 hours	2 hours	2 5 hours	3 hours	3.5 hours	4.5 hours		
per cent	per cent	°C.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
COMMERCIALLY DRIED WHOLE EGG											
95.74*	...	110	....	95.89	...	....	....	....	....	....	....
95.80*	96.97			95.93							
95.80	97.00	115	95.80* 95.80*	....	....	....	....	....	....	95.80	95.84
			95.84 95.84								
COMMERCIALLY DRIED EGG ALBUMEN											
94.66* 94.67*	...	110	....	94.70	....	....	....	....	....	....	....
94.67 94.68	95.71			94.72							
94.73*	95.91	115	94.55* 94.68*	...	...	...	...	...	...	94.39	94.46
94.78			94.70 94.74								
LIQUID WHOLE EGG											
SAMPLE A											
26.77	30.14	115	27.12	...	26.87	....	....	....	....	...	...
26.90	35.80		27.26		26.90						
26.90			27.30		27.05						
SAMPLE B											
26.10		115	26.72	...	26.13*	...	...	26.08*	...	....	....
26.10			30.76		26.26			26.11			
26.11			32.38		26.40						
..			...	....	26.17*	26.22	26.14	26.12*	26.14	26.18	26.20
					26.28	26.28	26.14	26.14	26.14	26.18	26.20
					26.32	26.32	26.14	26.14	26.14	26.18	26.20
						26.37	26.14	26.14	26.14	26.18	26.20

\* Obtained on different days.

## EXPERIMENTAL.

The table presents results obtained on samples of dried commercial egg yolk, dried whole egg, dried albumen, and liquid whole egg. For comparative purposes results obtained by a vacuum method<sup>1</sup> employing a temperature of 55°C. and a pressure of about 13 cm. mercury are in-

<sup>1</sup> U. S. Dept. Agr. Bull. 846, p. 89.

cluded. The dried egg samples were prepared for analysis by grinding in a hand mill and sifting through a household flour sifter. The liquid egg samples were prepared from shell eggs by mixing thoroughly with an egg beater to secure uniformity. The conditions for drying are indicated in the table. The bulk of the moisture of the liquid egg was removed by heating on the steam bath for 15-30 minutes before placing in the air oven, but this time is not included in the drying periods given in the table. Aluminum moisture dishes, diameter about 55 mm., height about 15 mm., and provided with slip-in inverted covers, fitting tightly on the inside, were used. Quantities equivalent to approximately 2 grams of egg solids were taken for the determinations.

### CONCLUSIONS.

Certain deductions may be drawn from the tabulated results. Commercially dried egg material, prepared as indicated above, when dried in an air oven at 110°-119°C. for a period of about 1 hour and liquid egg dried at 115°C. for a period of approximately 3 hours yield results for total solids closely consonant to those obtained by the umpire vacuum method. Slight variations in the drying periods do not affect the results. A temperature of approximately 115°C. for one hour, for commercially dried egg material, and three hours for liquid egg appears favorable for the desired routine method. The results obtained by such a method will not be affected by slight variations in time and temperature from those prescribed.

On the basis of the experimental data presented and of the deductions therefrom, the following rapid practical routine method is proposed for determining total solids in dried and liquid eggs.

### METHOD.

#### APPARATUS.

(a) *Metal dish*.—Diameter about 55 mm., height about 15 mm., provided with slip-in inverted cover fitting tightly on inside.

(b) *Air-tight desiccator*.—Should contain reignited quick lime or calcium carbide.

(c) *A drying oven*.—Should maintain a temperature of 112°-117°C. and be provided with an opening for ventilation. A thermometer resting in a beaker of mercury on the shelf holding the samples may be used for indicating the oven temperature.

#### DETERMINATION.

*Liquid egg*.—Weigh accurately approximately 5 grams of homogeneous sample in the covered dish, which has been dried at 112°-117°C., cooled in the desiccator, and weighed soon after attaining room temperature. Drive off the bulk of the water by heating on the steam bath for approximately 30 minutes. Continue the drying in the oven at 112°-117°C. for approximately 3 hours. Cover the dish, transfer to the desiccator, and weigh soon after room temperature is attained. Report the egg residue as total solids.

*Dried eggs*.—Use approximately 2 grams of the finely powdered, well mixed sample, accurately weighed. Follow the directions for liquid egg, omitting the preliminary drying on the steam bath. Dry for approximately 1 hour.

## A COMPARATIVE STUDY OF THE GUNNING-ARNOLD AND WINKLER BORIC ACID MODIFICATIONS OF THE KJELDAHL METHOD FOR THE DETERMINATION OF NITROGEN<sup>1</sup>.

By K. S. MARKLEY (Soil Bacteriology, Bureau of Plant Industry) and  
RAYMOND M. HANN<sup>2</sup> (Nitrogen Laboratory, Bureau of Chemistry,  
U. S. Department of Agriculture, Washington, D. C.).

In spite of the known accuracy of the boric acid absorption method, its advantages over the Kjeldahl-Gunning-Arnold method, and its availability for over ten years, it has received scant consideration in the nitrogen laboratories of this country. It is believed that owing to the absence of sufficient published data comparing the two methods, most workers have preferred to use the well-known modified Kjeldahl method.

It is proposed, therefore, to present these comparative data, give the details of the procedure, and emphasize again the advantages of the method. A number of distinct classes of nitrogenous compounds were chosen in order to cover as many different fields of nitrogen analyses as possible.

### BORIC ACID ABSORPTION.

L. W. Winkler<sup>3</sup>, in 1913, first proposed the substitution of boric acid for sulfuric acid for the fixation of the distilled ammonia. He absorbed the ammonia in a 5 per cent boric acid solution, and the ammonium borate thus formed was titrated with standard acid, either congo red or methyl orange being used as an indicator. No comparative results are included in these papers.

E. Bernard<sup>4</sup>, following the method as outlined by Winkler, applied it to the analysis of concentrated feeding stuffs. The results are equally as accurate as those obtained with the older method. Schulze<sup>5</sup>, in determining nitrates and nitrites in water, used boric acid for the absorption of the distilled ammonia with excellent results.

In 1916, Adler<sup>6</sup> modified the method in order to adapt it to the use of the brewing industry laboratories. He gave the results of numerous experiments, which may be briefly summarized as follows:

With a 4 per cent solution of boric acid as the fixing medium, he used as indicators methyl orange and congo red, finding the end point of the

<sup>1</sup> Presented at the 68th meeting of the American Chemical Society, Ithaca, N. Y., Sept. 8-13, 1924, and published here by courtesy of *Industrial and Engineering Chemistry*.

<sup>2</sup> The authors wish to express their thanks to F. L. Goll, of the Office of Soil Bacteriology, for photographs, sketch, and graphs contained in this paper; also to Agnes Quirk, of the Office of Plant Pathology, for the electrometric pH determinations.

<sup>3</sup> *Z. angew. Chem.*, 1913, 26: 231; 1914, 27: 630.

<sup>4</sup> *Ibid.*, 1914, 27: 664; *Landw. Vers-Sta.*, 1915, 86: 331.

<sup>5</sup> *Mitt. kgl. Landesanstalt Wasserhyg.*, 1914, 18: 87

<sup>6</sup> *Z. ges. Brauw.*, 1916, 39: 162 and 169.

latter not so sharp as the former, but he failed to state whether he titrated by natural or artificial light. He further showed that distillation was complete in 20 minutes, the final volume of the distillate being 220–250 cc. He did not give the blank obtained on the reagents used, but gave the correction to be subtracted from the buret reading due to the reaction of the water in the distillate. This correction, for 250 cc. of distilled water, he found to be 0.15 cc. of 0.1 *N* sulfuric acid and for 50 cc. boric acid solution + 200 cc. distilled water, 0.13 cc. of 0.1 *N* sulfuric acid. He further found that when the ammonia did not reach the boric acid at room temperature it was either not absorbed<sup>1</sup> or, if it were, the ammonium borate formed, being a weak salt, was decomposed by heat with a consequent loss of ammonia.

When a solution of ammonium chloride was used as the source of ammonia and methyl orange as an indicator, Adler obtained the following results:

NH <sub>4</sub> Cl SOLUTION USED	NITROGEN	
	Usual method	H <sub>3</sub> BO <sub>3</sub> method
cc.	mg.	mg.
10	20.56	20.62
20	40.98	40.93
30	61.36	61.25

He applied the method to the determination of protein in barley and malt with good agreement.

Scales and Harrison<sup>2</sup>, in this country, tested the method in 1920 with a view to adapting it to the analysis of crops and soils. They used ammonium sulfate as the source of ammonia and tried methyl orange, congo red, and bromphenol blue as indicators with boric acid. They used both sulfuric and boric acids for the absorption of the ammonia and showed that after correcting for the different indicators both methods gave practically identical results. They also presented data showing the agreement of triplicate analyses for three types of crops and of soil when using boric acid for fixation and bromphenol blue as indicator.

Spears<sup>3</sup>, in 1921, compared the boric acid absorption procedure with the modified Kjeldahl method in use at the Lexington, Kentucky, Agricultural Experiment Station, and although he found almost complete agreement in the analysis of a number of feeding stuffs he stated that in his opinion there was not much choice between the two methods.

<sup>1</sup> See also Kober and Graves, *J. Am. Chem. Soc.*, 1913, 35: 1594.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1920, 12: 350.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1921, 5: 105.

Boric acid absorption has been in use in the laboratory of the Office of Soil Bacteriology (Fig. 1) for a number of years and in one period of six months over 10,000 nitrogen analyses were made by one chemist with the help of an unskilled assistant. These analyses covered many types of crops including cereals, legumes, potatoes, etc., together with soil and bacterial cultures.

Since Kellner<sup>1</sup> showed that phosphoric anhydride or potassium sulfate may be used equally as well in the Kjeldahl digestion and Gibboney<sup>2</sup> reported the digestion of acid phosphate plus dried blood and of acid phosphate plus cottonseed meal by Fuller's copper sulfate method led to practically identical results with the official method, there seemed to be no reason for modifying the method of digestion practised in the two laboratories engaged in this investigation.

#### PROCEDURE USED WITH BORIC ACID ABSORPTION.

The procedure followed was that outlined by Scales and Harrison and is as follows:

Digest the samples of nitrogenous substances, other than nitrates, with 30 cc. of sulfuric acid to which has been added 10 per cent by weight of phosphoric anhydride and 0.5 gram of anhydrous copper sulfate<sup>3</sup>, for 1½–4 hours, depending upon the size of sample and the nature of the material. Cool, add 150–175 cc. of distilled water, make alkaline with sodium hydroxide, and distil into 50 cc. of 4 per cent boric acid solution containing 3 drops of bromphenol blue<sup>4</sup>. Distil until the total volume in the receiving flask is approximately 175 cc., lower the flask to allow washing down of the tube, and continue distillation to a volume of 200 cc. Titrate directly with *N*/14.01 sulfuric acid in the enclosed cabinet (Fig. 2) to the disappearance of the purple color. The acid is conveniently standardized against Bureau of Standards standard benzoic acid, phenolphthalein being used as the indicator.

#### KJELDAHL—GUNNING—ARNOLD METHOD.

The method now in use in the Nitrogen Laboratory of the Bureau of Chemistry, which was utilized in this investigation, is the official Kjeldahl-Gunning-Arnold method of the Association of Official Agricultural Chemists as adopted by that organization<sup>5</sup> and thereafter modified by Daudt<sup>6</sup> in order to reduce the volume of acid and the weight of alkali sulfate added, as well as to substitute mercuric oxide as a catalyst for the less efficient copper sulfate. An excellent review and study of the various modifications of this method is given by Paul and Berry<sup>7</sup>. Phelps and Daudt<sup>8</sup> show that mercuric oxide is more effective than copper sulfate, molybdic acid, titanous acid, vanadic acid, and other metallic catalysts in the hydrolysis of pyridine zinc chloride.

<sup>1</sup> *Landw. Vers.-Sta.*, 1902, 57: 15.

<sup>2</sup> *Proceedings Association Official Agricultural Chemists for 1906*, p. 76.

<sup>3</sup> Sulfuric acid containing  $P_2O_5$ , which is now sold in this country by the General Chemical Company, Easton, Pa., has long been used by most of the experiment stations in Germany, where in 1895 the use of this mixture was adopted as official. See reference 1 above.

<sup>4</sup> Dissolve one gram of bromphenol blue in 20 cc. of 0.05 *N* NaOH with gentle heat and dilute resultant solution to 1 liter.

<sup>5</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 7.

<sup>6</sup> *J. Assoc. Official Agr. Chemists*, 1921, 4: 366.

<sup>7</sup> *Ibid.*, 5: 108.

<sup>8</sup> *Ibid.*, 1920, 4: 72.

Briefly outlined, the method is as follows:

Digest the sample with 25 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate, and 0.7 gram of mercuric oxide for a period of 2 hours after the liquid becomes clear. Cool, dilute, precipitate the mercury as sulfide by the addition of thiosulfate solution, add a few particles of granulated zinc and an excess of sodium hydroxide, and distil the ammonia over through block tin condensers into a measured excess of standard acid. Following distillation, wash down the condensers and titrate back the excess acid with standard alkali, using sodium alizarin sulfonate indicator.

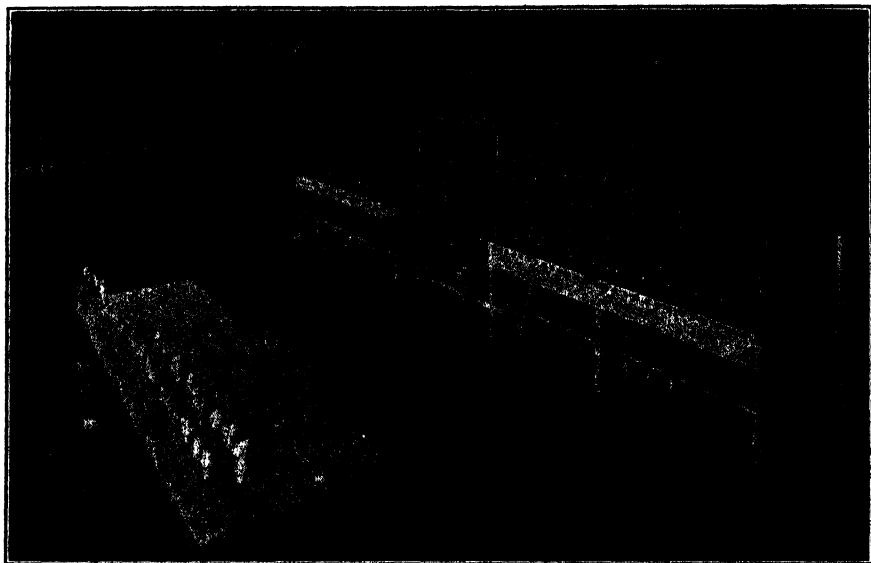


FIG. 1.—KJELDAHL OUTFIT ARRANGED TO HANDLE SIMULTANEOUSLY 60 DIGESTIONS AND 40 DISTILLATIONS USING THE BORIC ACID METHOD.

## RESULTS.

Table 1 gives the results of the analyses of a number of addition compounds of halogen-o-toluidines prepared and purified by one of the writers during another investigation for the Bureau of Chemistry<sup>1</sup>.

For the substances shown in Table 2 both methods were further modified so as to include nitrate nitrogen. In both cases 33½ grams of salicylic acid was added per liter of sulfuric acid; with the boric acid method reduction was furthered by the addition of one gram of zinc dust and with the Kjeldahl-Gunning-Arnold method by the addition of 5 grams of dry sodium thiosulfate, the samples thus treated being allowed to remain at room temperature one-half hour before the digestion was begun.

<sup>1</sup> Hann and Spencer. The Addition Compounds of Dibromo-o-Toluidines with Metallic Salts. *J. Wash. Acad. Sci.*, 1925, 15: 163.

Table 3 gives comparative results of some proteins and substances containing protein.

All the compounds shown in Tables 4 and 6 were purchased chemicals and were not repurified before analysis.

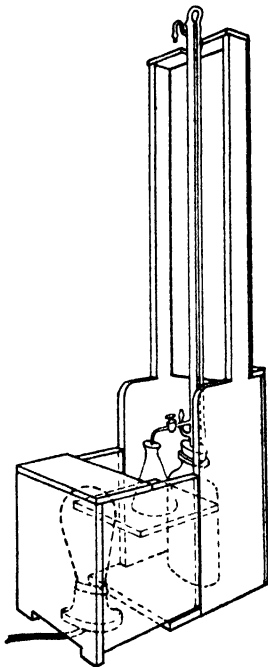


FIG. 2.—CABINET WITH ARTIFICIAL LIGHT SOURCE FOR TITRATING AMMONIA.

Of the heterocyclic compounds shown in Table 5, all except isatin and indigo carmine were crude substances analyzed during preparation to determine their purity. The two excepted compounds were purchased reagents.

With none of the substances shown in Tables 1-5 was difficulty experienced in obtaining agreement with the two methods when digestion was carried on for 2-3 hours<sup>1</sup>. However, with the alkaloids shown in Table 6 difficulty was experienced from the outset in obtaining satisfactory agreement.

<sup>1</sup> Similar close agreement has been recorded by Kellner (*Landw. Vers.-Sta.*, 1902, 57: 297), in a report based on comparative tests made by six German experiment stations. Concentrated feeding stuffs were used for these tests.



TABLE 1.  
*Addition compounds of halogen-o-toluidines.*

NAME OF COMPOUND USED	THEORETI- CAL NITROGEN	BORIC ACID ABSORPTION					K-G-A. METHOD				
		Quantity of substance used	N/14H <sub>2</sub> SO <sub>4</sub> used for titration	Reagent blank N/14H <sub>2</sub> SO <sub>4</sub>	Actual quantity required to neutralize NH <sub>3</sub>	Nitrogen found	Quantity of substance used	0.1 N HCl used for titration	Reagent blank 0.1 N HCl	Actual quantity 0.1 N HCl required to neutralize NH <sub>3</sub>	Nitrogen found
Dibromo-o-toluidine CdCl <sub>2</sub> , 2 : 1	per cent 3.93	gram 0.2603	cc. 11.53	cc. 0.67	cc. 10.86	per cent 4.17	gram 0.1498	cc. 4.8	cc. 0.4	cc. 4.4	per cent 4.11
		0.23115	10.38	0.67	9.71	4.20	0.1484	4.6	0.4	4.2	3.96
					Average	4.18				Average	4.08
Dibromo-o-toluidine ZnCl <sub>2</sub> , 2 : 1	4.21	0.3482	15.20	0.67	14.53	4.17	0.3325	10.5	0.4	10.1	4.25
		0.2675	11.92	0.67	11.25	4.21	0.2116	6.5	0.4	6.1	4.03
					Average	4.19				Average	4.14
Dibromo-o-toluidine CdBr <sub>2</sub> , 2 : 1	3.49	0.2393	9.25	0.58	8.67	3.62	0.1290	3.5	0.4	3.1	3.36
		0.2307	8.58	0.58	8.00	3.47	0.1373	3.9	0.4	3.5	3.57
		0.21735	8.39	0.58	7.81	3.64	0.1255	3.6	0.4	3.2	3.57
					Average	3.58				Average	3.49
Iodo-o-toluidine HgCl <sub>2</sub> , 2 : 1	3.61	0.11865	4.90	0.68	4.22	3.59	0.1293	3.7	0.4	3.3	3.57
							0.1275	3.7	0.4	3.3	3.62
										Average	3.59
3,5-Dibromo-o-toluidine HgCl <sub>2</sub> , 2 : 1	3.50	0.1534	5.80	0.68	5.12	3.34	0.1003	2.8	0.4	2.4	3.35

TABLE 2.  
*Soil, peat, and manure\*.*

SUBSTANCE UNDER ANALYSIS	SOURCE OF MATERIAL	BORIC ACID ABSORPTION		K.-G.-A. METHOD	
		No. of samples	Nitrogen found	No. of samples	Nitrogen found
Greenhouse soil	Arlington, Va.	3	<i>per cent</i> 0.103	3	<i>per cent</i> 0.103
Muck soil	Arlington, Va.	3	2.55	3	2.60
Loamy soil	Champaign, Ill.	4	0.360	3	0.363
Sedge peat	Corona, Minn.	3	2.06	3	2.11
Leached horse manure		4	0.952	3	0.962

\* All results reported on air-dry basis.

TABLE 3.  
*Protein and substances containing protein.*

SUBSTANCE UNDER ANALYSIS	BORIC ACID ABSORPTION		K.-G.-A. METHOD	
	NO. OF SAMPLES	NITROGEN FOUND	NO. OF SAMPLES	NITROGEN FOUND
Cottonseed meal	4	<i>per cent</i> 6.80	4	<i>per cent</i> 6.78
Dried blood	2	14.02	3	14.03
Corn (whole plant)	3	0.60	3	0.61
Peas (whole plant)	3	2.74	3	2.75
Mucine (from bile)	3	0.43	1	0.38
Egg albumin	4	12.99	3	12.78
Pancreatin	3	9.26	2	9.13

There was a difference, apparently, in the rate at which the catalysts used in the two methods attacked the ring structures that was not apparent with the straight chain compounds nor with the heterocyclic compounds recorded in Table 5.

To obtain some idea of the relative rates of digestion, the following procedure was adopted: Eighteen burners were adjusted to bring 250 cc. of water to the boiling point in 10 minutes. Eighteen samples of 0.2 gram each of quinoline methiodide were weighed. They were treated with sulfuric acid containing in one case mercuric oxide-potassium sulfate and in the other copper sulfate-phosphoric anhydride, as described

TABLE 4.  
*Amino acids, amines, amides, and imino bodies.*

COMPOUND		NITROGEN THEORY	BORIC ACID ABSORPTION		K.-G.-A. METHOD	
Name	Class		No. of samples	Nitrogen found	No. of samples	Nitrogen found
Leucin	Mono amino acid	<i>per cent</i> 10.69	2	<i>per cent</i> 10.47	2	<i>per cent</i> 10.68
Cystine	Diamino acid	11.66	3	11.34	1	11.29
Beta-amino Anthraquinone	Amino body	6.28	1	6.26	2	6.27
Amino-phenol (meta)	Amino body	12.84	3	12.78	3	12.57
Cumidine (pseudo)	Primary amine	10.37	3	10.30	2	10.19
Succinimide	Imino body	14.14	3	13.90	2	13.85
Asparagine	Amino-acid amide	21.21	3	19.95	1	19.99

TABLE 5.  
*Heterocyclic nitrogen compounds.*

NAME OF COMPOUND (CRUDE SUBSTANCES)	NITROGEN, THEORY	BORIC ACID ABSORPTION		K.-G.-A. METHOD	
		No. of samples	Nitrogen found	No. of samples	Nitrogen found
IsatinyI-p-tolyl rhodanic acid	<i>per cent</i> 8.43	2	<i>per cent</i> 7.79	2	<i>per cent</i> 7.81
2 thio-3-p-tolyl-5-iodovanillyl-4- thiazolidone	3.14	2	2.88	1	2.83
Bromomethyl piperdyl-delta <sup>2</sup> - thiazoline	10.64	2	7.74	2	7.84
2-(beta-furfuryl)-vinyl-quinoline- methiodide	3.86	2	3.18	3	3.25
Isatin	9.53	1	9.13	1	9.08
Indigo carmine	6.01	2	4.78	1	5.02

TABLE 6.  
*Alkaloids.*

NAME OF COMPOUND	NITROGEN, THEORY	BORIC ACID ABSORPTION		K.-G.-A. METHOD	
		No. of samples	Nitrogen found	No. of samples	Nitrogen found
Heroin	<i>per cent</i> 3.80	2	<i>per cent</i> 3.54	2	<i>per cent</i> 3.56
Cinchonine	9.52	2	9.33	2	9.36
Strychnine sulfate	6.54	3	6.51	2	6.47
Caffeine	28.87	2	28.02	2	28.08
Cocaine hydrochloride	4.12	3	3.95	1	4.08

previously. Every 5 minutes up to 45 minutes two samples were removed from the digestion rack and distilled according to the Kjeldahl-Gunning-Arnold method. In both series considerable carbonaceous matter was left at the end of 5 minutes, and no nitrogen was found in the distillate. At the end of 10 minutes both methods showed clear liquid with 7.04 per cent and 5.63 per cent of the *N* with copper sulfate-phosphoric anhydride and mercuric oxide-potassium sulfate, respectively.

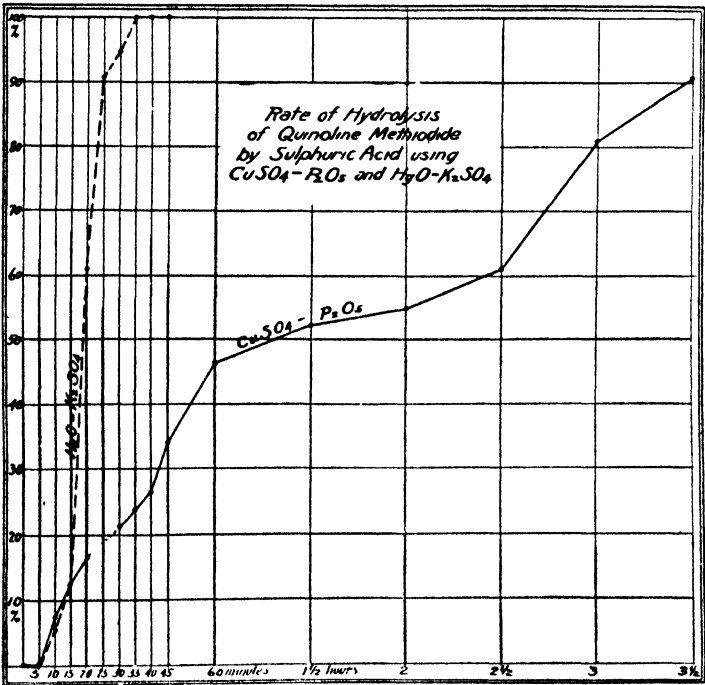


FIG. 3

It will be noticed from Table 7 and the graph (Fig. 3) that 100 per cent of the nitrogen was recovered at the end of 35 minutes with mercuric oxide-potassium sulfate, while only 23.95 per cent was recovered with copper sulfate-phosphoric anhydride. Digestion and removal of samples at half-hour intervals from 1-3½ hours was continued in the case of copper sulfate-phosphoric anhydride with the result that at the end of 3½ hours only 91.4 per cent of the nitrogen was recovered.

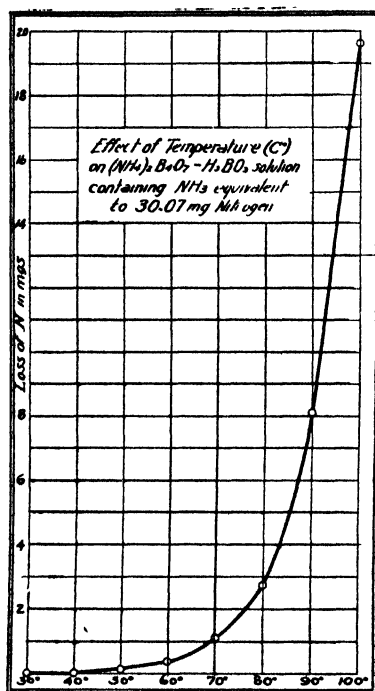


FIG. 4.

This experiment was not continued beyond 3½ hours but, as shown in Table 6, digestion for 4 hours with copper sulfate-phosphoric anhydride gives results for alkaloids that agree well with those obtained by using the mercuric oxide-potassium sulfate.

#### EFFECT OF TEMPERATURE ON AMMONIUM BORATE.

Since Adler warns against the use of air-cooled condensers, common in German nitrogen laboratories, when using the boric acid method, a few experiments were made to determine at what temperature and how rapidly ammonium borate was decomposed under the conditions obtaining in the receiving flasks.

TABLE 7.

*Rate of hydrolysis of quinoline methiodide by sulfuric acid using  $\text{CuSO}_4\text{-P}_2\text{O}_5$  and  $\text{HgO-K}_2\text{SO}_4$ .*

SAMPLE NO.	TIME	$\text{CuSO}_4\text{-P}_2\text{O}_5$		$\text{HgO-K}_2\text{SO}_4$	
		Nitrogen distillate	Nitrogen recovered	Nitrogen distillate	Nitrogen recovered
	minutes	per cent	per cent	per cent	per cent
1	5				
2	10	0.305	7.04	0.28	5.63
3	15	0.63	12.67	0.63	12.67
4	20	0.84	16.90	3.08	61.97
5	25	0.77	15.49	4.55	91.54
6	30	1.05	21.12	4.76	95.77
7	35	1.19	23.95	4.97	100.00
8	40	1.33	26.75	4.97	100.00
9	45	1.70	34.20	4.97	100.00
10	60	2.31	46.47		
11	90	2.59	52.11		
12	120	2.73	54.92		
13	150	3.08	61.95		
14	180	4.06	81.69		
15	210	4.55	91.40		

Twenty cc. of ammonium hydroxide solution, equivalent to 30.07 mg. of nitrogen, were added to 50 cc. of 4 per cent boric acid, diluted to 200 cc., heated to various temperatures for thirty minutes, cooled to room temperature, and titrated. The loss of nitrogen is shown graphically in Fig. 4. It will be noticed that up to approximately 50°C. no appreciable loss occurs, but at 60 degrees the loss is 1.29 per cent and at 100°C. 65.25 per cent. Heating of the liquid in the receiving flask above 50° by radiation from gas burners is therefore to be avoided to obtain maximum accuracy. Tests made with the apparatus shown in Fig. 1 indicated, however, that even with high outside temperature and all forty burners in use, the temperature in the receiving flasks did not exceed 40°C.

#### DISCUSSION OF INDICATORS.

As shown in Table 1 the blanks are higher with the boric acid than with the Kjeldahl-Gunning-Arnold method, which is due in part to the difference in concentration of the acids used for titration and in part to the indicators. Alizarin sodium sulfonate<sup>1</sup> or alizarin red S-780 (Schultz), the indicator used with the Kjeldahl-Gunning-Arnold method, has an effective pH range of 5.0-6.8 (yellow-pink), which includes the pH of the distilled water in the laboratory. Less than 0.05 cc. of 0.1 *N* hydrochloric acid is required to bring the water to alizarin sodium sulfonate neutrality, thereby contributing practically nothing to the blank. On the other hand, the indicator used with the boric acid method, brom-

<sup>1</sup> Clark. *The Determination of Hydrogen Ions*, 1922 ed., p. 91.

phenol blue, has an effective pH range approximately between 3.0 and 4.6. When the bromphenol blue indicator is added to 200 cc. of distilled water it requires about 0.30 cc. of  $N/14.01$  acid to cause the blue color to disappear or to bring the water to pH 4.0, which is taken as the bromphenol blue neutrality.

When 50 cc. of 4 per cent boric acid is placed in the receiving flask, no blue color is developed on the addition of the indicator, the solution being practically neutral to bromphenol blue, but on dilution to 200 cc. the blue color is immediately developed and requires, as with distilled water alone, approximately 0.30 cc.  $N/14.01$  sulfuric acid to cause it to disappear. A difference of as much as 50 cc. more or less of water has no appreciable effect on the quantity of acid required to cause the blue color to disappear. Scales and Harrison found it necessary to subtract 0.35 cc. from the buret reading of the sulfuric acid to correct, as they state, for the presence of the boric acid, which in reality is due to the water, as is shown in Table 8.

TABLE 8.  
*Effect of indicator on  $H_2O$  alone and on diluted boric acid solution.*

SOLUTION	N/14.01 $H_2SO_4$ REQUIRED TO BRING SOLUTION TO B. P. B. NEUTRALITY*	pH DETERMINED WITH HYDROGEN ELECTRODE, TEMPERATURE 26°C.	
		Before neutralization	After neutralization
200 cc. distilled $H_2O$	cc 0.30	pH 5.23	pH 4.07
50 cc. 4 per cent $H_3BO_3$ diluted to 200 cc.	0.26	4.60	4.06
50 cc. 4 per cent $H_3BO_3$ diluted to 250 cc.	0.28	4.92	4.09

\* Average of 5 determinations.

It will be noticed in the abstract of his work given previously that Adler found a similar effect when using methyl orange and properly attributed it to the reaction of the water.

From a consideration of the dissociation constant of boric acid ( $6.5 \times 10^{-10}$ ) it is to be expected that dilution of 50 cc. of 4 per cent boric acid solution to 200 cc. would not cause ionization sufficient to affect the titration.

Since both the blanks and the substance under analysis deliver the same quantity of liquid to the receiving flask the error, if it may be called such, is compensating

In the light of the above discussion of the pH effect on the determination of ammonia, it has been suggested that an error might be introduced by standardizing the sulfuric acid solution, using phenolphthalein whose

pH range is between 8.3–10.0 (Sorensen), and then using it with bromphenol blue whose pH range is roughly 3.0–4.6. However, it has been found by repeated standardization that whether or not the approximately  $N/14$  sodium hydroxide solution is titrated, *without further dilution*, with the  $N/14.01$  sulfuric acid, and phenolphthalein or bromphenol blue used as indicator, the value of the sulfuric acid in terms of sodium hydroxide is identical, and no error occurs in the determination of the nitrogen, as is shown in the comparative tables.

### CONCLUSIONS.

Extensive tests made with boric acid absorption have shown that it is as accurate as the Kjeldahl-Gunning-Arnold official method and deserves recommendation, provided water-cooled condensers are used and care is taken that the temperature in the receiving flask does not exceed 50°C.

Its advantages over the official method are:

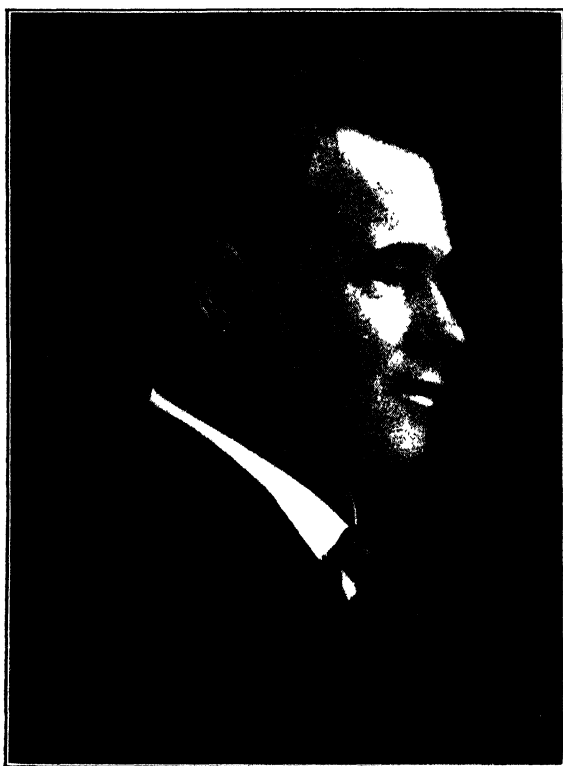
(1) The constant use of a standard alkali is eliminated with the attendant checking necessary owing to a changing alkalinity.

(2) Careful measurement of the fixing medium is not necessary. If for any reason the fixing medium is sucked back into the distilling flask, no damage is done. More boric acid can be added to the receiving flask and the distillation continued.

(3) Bromphenol blue may be used in artificial light, thus permitting titration to the same end point irrespective of varying light conditions in the laboratory.

In the course of these tests the efficiency of mercuric oxide-potassium sulfate and copper sulfate-phosphoric anhydride has been compared, with the result that both combinations have given satisfactory results with a large number of nitrogenous compounds. With certain nitrogenous compounds, especially with alkaloids, copper sulfate-phosphoric anhydride combination necessitates longer digestion; in other cases it acts as rapidly as the mercuric oxide-potassium sulfate. The use of the copper sulfate offers the advantage that no catalyst need be rendered inoperative before distillation.





EDWARD GEORGE PROULX, 1880—1925

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## EDWARD GEORGE PROULX

The sudden and untimely passing of Edward G. Proulx, State Chemist and Seed Commissioner of Indiana, was announced in the "Lafayette Journal and Courier" of April 1, 1925. His death occurred at the St. Elizabeth Hospital, Lafayette, Indiana, at 11:25 o'clock on the preceding evening, and although he had been in declining health for about two years, his demise was a great shock to his family and his many friends. He was apparently gaining strength after a protracted stay in the hospital at Indianapolis, begun last January, when in the discharge of his duties he contracted a cold from which he never recovered.

Mr. Proulx was born at Hatfield, Massachusetts, during the year of 1880 and upon the 8th of December, which places his span of life slightly over 44 years. He was married to Gertrude Nagle September 2, 1909.

His early collegiate studies were begun and completed at the Massachusetts Agricultural College, Amherst. His alma mater conferred the degree of Bachelor of Science upon him in 1903. Later, while serving as a member of the staff of the State Chemist Department of Purdue University Experiment Station, he completed post graduate studies at the University of Purdue and was granted the degree of Master of Science in 1909.

His first association with the work in which he was later destined to achieve coveted recognition was with the Fertilizer Control Department of the Massachusetts Agricultural Experiment Station. He served with Mr. Haskins, Official Chemist, for a period of three years dating from the Fall of 1903. In the early part of 1907 he transferred his activities to Purdue. In the reorganization of the State Chemist Department in 1916 he was placed in charge of the Fertilizer Division, and upon the death of W. J. Jones, Jr., State Chemist, this service culminated in his appointment as Acting State Chemist. In September, 1918, just a year later, his demonstrated ability as an executive resulted in his being raised to the status of State Chemist. When the State Seed Law was passed it brought added honors and responsibilities in the duties of Seed Commissioner.

He first attended the meetings of the Association of Official Agricultural Chemists during the Thirty-fourth Convention and was present at all subsequent meetings. Although this was his first official connection with the association, prior to this time he served frequently as a collaborator. In 1919, at the Thirty-fifth Convention, he was appointed to serve on the Committee on Sampling of Fertilizers, and again in 1923, he served upon the Committee on Definitions of Terms and Interpretation of Results, retaining this appointment until the time of his death.

In the Association of United States Feed Control Officials Mr. Proulx was particularly active from the time of assuming office in Indiana until recently. His constructive work in the numerous committees upon which he served, many of which were under his direction, lead to his being honored with the position of president of that association in 1924, and finally we find him faithfully serving upon the Executive Committee.

Mr. Proulx was also a member of the American Chemical Society, and his membership in the Association of Official Seed Analysts of North America was continuous from his assumption of official duties.

In civil life Mr. Proulx was accorded further honors. He was a member of the Rotary Club of Lafayette, only relinquishing membership shortly before his death, because of ill health. He joined the Masonic order at Amherst, Mass., retaining his affiliation up to the last. He enjoyed, also, the privilege of membership in the Lafayette Country Club.

Marked success was attained by Mr. Proulx in the administration of his official duties under the trying conditions prevailing during the World War. The extent of this achievement may be more correctly judged when we recall that the excessive demands of the war took many of his men who had become almost invaluable to the proper coordination of the work through long years of training. No doubt the loyal support of his staff was very helpful, but herein lies revealed the leadership of men that so strongly characterized Proulx, the man. Director Christie portrayed so aptly these qualities in a brief memoriam at the time of Mr. Proulx's death that it is reproduced in full:

“ ‘Success, when you sum it all up, isn't gold,  
Nor does it consist of deeds that are bold.  
The money we make and the houses we build  
Mean nothing the moment our voices are stilled.  
They live most who when they are gone  
In the lives of others are still living on’.

“We are met here to pay tribute to the memory of our associate and friend, Edward George Proulx.

“It is fitting that we should pause to consider the ideals and purposes which have endeared him to us and which are a living part of his work.

“Mr. Proulx made and held friends, and they are many. To his intimate associates he was Eddie Proulx, or just ‘Eddie’ and this explains how truly affable, approachable, and sincerely friendly he was. He will thus be remembered as a friend, modest, sincere, and helpful.

“But Eddie Proulx was more than a friend. He was a chemist of high rank. He was an efficient member of the Experiment Station family—a faithful and fearless executive, hewing to the line in the discharge of his official duties which were often arduous and difficult in the administration of the control laws with which he was entrusted.

“Mr. Proulx was also a leader of men. Although requiring faithful and efficient service of the members of his working staff, his honesty, sincerity, and kindly unostentatious bearing won the loyalty and support of his subordinates. His administration as State Chemist and Seed Commissioner, covering a period of eight years, will be remembered as one of progress and efficiency.

“Our brother has fallen but his good work will endure, and he will be remembered not only as a true friend but also as a fellow worker who wrought faithfully and well.

“In the passing of Mr. Proulx Purdue University sustains a great loss but may we not think of him as expressed in the lines of James Whitcomb Riley:

“ ‘A good man never dies—  
In worthy deed and prayer  
And helpful hands, and honest eyes,  
If smiles or tears be there:  
Who lives for you and me—  
Lives for the world he tries  
To help—he lives eternally.

Who lives to bravely take  
His share of toil and stress,  
And, for his weaker fellows' sake,  
Makes every burden less,—  
He may, at last, seem worn—  
Lie fallen—hands and eyes  
Folded—yet, though we mourn and mourn,  
A good man never dies' ”

Those who were intimately associated with Mr. Proulx speak of him with deep and sincere conviction. His bearing always produced a favorable impression. He was generous to a fault, always willing and eager to bear more than his share of the battle, whatever it was. Throughout the fifteen years which it was my privilege to know him he proved a true and loyal friend.

He leaves his widow, a mother, two sisters, and a brother to mourn his loss. His life so well spent in the service of others is the richest legacy he could leave to those most dear to him.

R. B. DEEMER.

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## ANNOUNCEMENT TO SUBSCRIBERS.

For some years the volume numbers of *The Journal* have not run with the calendar year, the first number of a given volume being issued in August of one year and the fourth, or last, in May of the following year. It has been decided to make the volume numbers coincident with the calendar year so that the complete proceedings of any regular annual meeting of the association may be found in one volume, the four numbers of which would issue during the calendar year immediately following that meeting.

The best way to bring this change about appears to be to put out the August and November 1925 issues as extra numbers (5 and 6) of Volume VIII and make the February 1926 issue No. 1 of Volume IX, as shown below:

Issues of. . . . .	May 1925	Aug. 1925	Nov. 1925	Feb. 1926
Old order. . . . .	Vol. VIII No. 4	Vol. IX No. 1	Vol. IX No. 2	Vol. IX No. 3
New order. . . . .	Vol. VIII No. 4	Vol. VIII No. 5	Vol. VIII No. 6	Vol. IX No. 1

Volume VIII, therefore, will contain six numbers, and binding should be deferred until the two extra numbers have been received. The index will appear in No. 6.

R. W. BALCOM,  
R. B. DEEMER,  
W. F. HAND,  
R. E. DOOLITTLE,  
H. D. HASKINS,  
*Board of Editors.*

## FIRST DAY.

### MONDAY—AFTERNOON SESSION—Continued.

#### REPORT ON SULFUR AND PHOSPHORUS IN THE SEED OF PLANTS<sup>1</sup>.

By W. L. LATSHAW (Agricultural Experiment Station, Manhattan, Kans.), *Associate Referee*.

The object of the work for 1924 was to secure additional information as to the reliability of the magnesium nitrate method for determining the sulfur and phosphorus in the seed of plants. Samples of cottonseed meal, soy bean meal, and mustard seed meal, representing a portion of the samples used in last year's work<sup>2</sup>, were used for analyses.

The reagents and details of oxidation and solution have been published<sup>2</sup>.

The following collaborative results on the determination of sulfur and phosphorus, using the magnesium nitrate method, were obtained:

COLLABORATOR	MATERIAL	SULFUR	PHOSPHORUS
		<i>per cent</i>	<i>per cent</i>
R. W. Titus Manhattan, Kans.	Cottonseed meal	0.51	0.92
		0.50	0.91
		0.54	
	Soy bean meal	0.39	0.54
		0.39	0.55
	Mustard seed meal	0.89	1.14
		0.91	1.12
J. F. Merrill Manhattan, Kans.	Cottonseed meal	0.52	0.89
		0.51	0.88
	Soy bean meal	0.38	0.52
		0.39	0.52
	Mustard seed meal	0.89	1.12
		0.88	1.13
W. L. Latshaw	Cottonseed meal	0.52	0.90
		0.52	0.89
	Soy bean meal	0.39	0.52
		0.41	0.53
	Mustard seed meal	0.90	1.14
		0.88	1.14

#### DISCUSSION.

The results of this year's work are offered as additional evidence in

<sup>1</sup> Presented by A. J. Patten.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 414.

favor of the magnesium nitrate method for the determination of sulfur and phosphorus in plants and in the seed of plants.

The results are extremely uniform, the duplication is excellent, and when compared with the figures reported last year on an analysis from a portion of the same sample, the same consistency and regularity is observed.

A word of caution is offered concerning the strength and quantity of magnesium nitrate used. A smaller quantity than prescribed is almost sure to result in a loss of sulfur. If it is found necessary to use a larger sample than prescribed, a proportionally greater quantity of the nitrate should be used.

Difficulty has been experienced in securing magnesium oxide or nitrate free of sulfur and phosphorus. This difficulty may be readily obviated by recrystallizing the salt, a very simple procedure. If care is used in recrystallizing, the first crop of crystals will be extremely free from sulfur, phosphorus, and other impurities.

As this is a report for the year, it is believed that it is not out of order to mention the seeming lack of interest among chemists in the work of the association. The referee trusts that other referees have not experienced the difficulties he has, which are somewhat as follows:

The secretary sent a list of eight or ten names of chemists that had expressed a desire to collaborate. A letter was written to each chemist, outlining the work planned for the year, which required less than four hours. All but two of the replies were to the effect that sufficient time was not available for any additional work. Samples were then mailed to the two that had expressed a willingness to collaborate. Early in September a call was made for the results. One replied that he was unable to perform the work on account of lack of time; the other was unable to secure magnesium nitrate of sufficient purity. The outcome of the year's work has been the results secured by the referee and two men in his laboratory.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the magnesium nitrate method for the determination of sulfur and phosphorus in plants including the seed of plants as outlined<sup>2</sup> be adopted as an official method.

(2) That the magnesium nitrate method take the place of the present official method.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 8: 263.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 414.

## REPORT ON DAIRY PRODUCTS.

By JULIUS HORTVET (Minnesota State Dairy and Food Department, St. Paul, Minn.), *Referee*.

Following the adoption of the Babcock method (first action) as an official method in the form submitted by the referee at the meeting of this association last November, it may be recalled that four recommendations were approved, among which was the following:

(3) That the proposed changes and amendments in the Babcock method be subjected to collaborative study and investigation by the referee.

After the approval of the recommendations, four proposals embodied in a motion offered by W. W. Randall were favorably acted upon. The referee was instructed to canvas the views of dairy science experts relative to certain proposed changes in and amendments to the official Babcock method. Acting accordingly, within a few weeks following the last meeting, a questionnaire was prepared embodying the proposals contained in the Randall motion. A copy of the questionnaire, with accompanying letter of explanation, was submitted to each of 30 individuals whose names appear prominently in the membership of the A. O. A. C. and the A. D. S. A. as experts presumably interested in the study of methods of analysis for dairy products. It was not believed necessary to extend the list indiscriminately to include persons who, while practically trained in the operation of the Babcock test, might not be likely to possess any definite technical knowledge regarding the subjects under discussion.

In due time the views of twenty persons were obtained, and the results are herewith summarized under headings conforming with the language of the original proposals:

(1) The need for a standard 30 per cent, 9 gram, short-neck, 6 inch cream-test bottle to supplement the several cream-test bottles already specified.

The result of the vote on the above proposition indicates 3 persons in favor of adoption and 14 opposed.

(2) The desirability of demanding a greater accuracy of graduation, in the case of the 50 per cent, long-neck, 9 inch cream-test bottles, than has been specified.

The result of the vote on the above proposition indicates 8 persons in favor of adoption and 12 opposed.

(3) The desirability of providing for a milk pipet, similar in every respect to the one specified, except that it shall be graduated to *deliver* 17.45 cc. of water at 20°C. when drained in the manner prescribed by the Bureau of Standards for transfer pipets.

The result of the vote on this question indicates 4 persons in favor of adoption and 15 opposed.

(4) The desirability of providing that the 18 gram charge of milk may be *weighed* into the test bottle instead of being measured by means of the pipet.

The result of the vote on this question indicates 5 persons in favor of adoption and 15 opposed.



Considerable discussion relating to some of the subjects covered by the questionnaire was submitted, but it is not deemed necessary to include in this report any quotations from the correspondence. Regarding the milk pipet particularly, views expressed are only repetitions, pro and con, of the arguments that were outlined in the report of the referee a year ago.

**REPORT OF COMMITTEE ON STANDARDIZATION OF EQUIPMENT OF INTERNATIONAL ASSOCIATION OF MILK DEALERS.**

(1) We see no need of the addition of another bottle, to the already existing equipment of bottles in that class.

(2) Whereas the volume of the smallest unit of graduation represents one-half of one per cent in this particular bottle, the requirement of greater accuracy would add practically nothing to the general accuracy of the work, and would also probably increase the cost of these bottles unnecessarily.

(3) We do not think that the graduation on a milk pipette should be changed from 17.6 cc., for the reason that these are almost universally used, and what becomes universal usage sets a precedent for universal law, and if another pipette were permitted, comparisons of results of the two (2) pipettes might result in a difference, whereas if all are using the one common measure, there would be no difficulty on this score.

(4) While technically speaking, an 18 gramme charge of milk weighed into a test bottle would be scientifically correct, yet in view of the fact that practically 100 per cent of the users of the Babcock test use the 17.6 pipette, and as it is found sufficiently accurate for all practical and commercial purposes, we would not deem it wise to permit any other method of measurement, except for exceedingly delicate work in very fine computations, for scientific purposes.

Very truly yours,

INTERNATIONAL ASSOCIATION OF MILK DEALERS.

By R. E. LITTLE (Signed),

Secretary.

Chicago, Illinois, August 13, 1924.

**REPORT OF AMERICAN DAIRY SCIENCE ASSOCIATION COMMITTEE ON OFFICIAL METHODS FOR TESTING MILK AND CREAM FOR BUTTERFAT.**

*Extract from committee report.*

Your committee has carefully considered the suggestions contained in Randall's motion and beg to submit the following recommendations:

1.—That this association does not favor the recognition of the 30 per cent 9 gram short-neck test bottle as an additional standard bottle to supplement the three standard cream test bottles already adopted. This bottle was carefully considered by the American Dairy Science Association at the time the specifications for standard Babcock glassware were assembled, and it was then decided that the three standard cream test bottles adopted fulfilled the requirements of all important branches of the industry.

One of the important purposes of standardizing Babcock test bottles obviously was and still is to reduce the number of different types of bottles to a practical minimum. When this work was first undertaken some 14 years ago, there were no less than 27 different types of test bottles on the market. In this process of elimination we endeavored to select as standard bottles an assortment of types that would most adequately

take care of the diversified demands of the different branches of the industry. Thus, for instance, in order to meet the demand of those branches and laboratories that required a test of relatively extreme accuracy we adopted the 9 gram 9 inch 50 per cent bottle and the finest graduation of this bottle is very similar to that of the 30 per cent 9 gram bottle.

There is no objection to the adoption of the 30 per cent 9 gram bottle provided that the industry demands and is in need of such a bottle. Outside of its mention in the discussion of our conference of September 14 and 15, 1923, the need of the 30 per cent bottle has never been called to our committee's attention. Louis F. Nafis, Inc., manufacturers of Babcock glassware, claim that they have no call for 30 per cent bottles. They further advise us that they are receiving some inquiries for a 20 per cent bottle for testing ice cream and for a 55 and 60 per cent cream test bottle. These non-standard bottles are used to some extent unofficially and there is no objection thereto.

On the basis of our present information we feel that there is not sufficient evidence of a demand for the 30 per cent bottle to justify official recognition of this bottle.

2.—That we do not favor any change in the requirements for tolerance or limit of error in the case of the 50 per cent long neck 9 inch bottle at this time.

We are in full accord with all efforts leading towards maximum accuracy of standard Babcock glassware and we believe it desirable to have each piece of apparatus and every phase of the method of operation as nearly perfect as possible. There is, however, a limit beyond which perfection is no longer practical. It is our judgment that this limit is set by the smallest division in the graduation, and we further believe that any attempt at this time at a tolerance smaller than the smallest division in the graduation would not add materially to the accuracy of the test, while it would considerably increase the cost to the user of such glassware. It would place an extra burden on the industry without insuring tangible benefit. It is our judgment, therefore, that the limit of error, as prescribed by the specifications of the A. D. S. A. and the U. S. Bureau of Standards, should not be changed.

3.—That we do not favor the substitution nor the supplementation of the adopted standard milk pipette that is graduated to contain 17.6 cc. by a pipette graduated to deliver 17.45 cc.

We fail to see any tangible advantage in making this change and fear that such substitution or supplementation would be a cause of confusion to the industry that has become used to the pipette as it now is and as it has been approved by the U. S. Bureau of Standards.

4.—That we do not favor supplementing our standard directions for testing milk by a clause providing that the 18 gram charge of properly prepared milk may be weighed into the test bottle instead of being measured by means of the pipette.

There can obviously be no objection to weighing instead of measuring the milk into the test bottle and this may be safely left to the option of the operator without the necessity of complicating the standard directions by incorporating this alternative.

Approved.

Submitted by Committee on Official Methods for Testing Milk and Cream for Butterfat at the annual meeting of American Dairy Science Association, Milwaukee, Wis., September 29, 1924.

The conclusion is, therefore, justifiable, in respect to all of the proposed changes in Babcock glassware, that no action is called for on the part of the Bureau of Standards and that no amendments to the present method are to be recommended for adoption by this association. Respecting the fourth suggestion favoring the alternative method of weighing the sample of properly prepared milk instead of measuring by means

of the pipet, there turns out to be also an overwhelming negative expression of views, and as a result no recommendation can be made favoring the adoption of this proposed change.

It is timely, during the present meeting, that attention be devoted to certain chemical and physical methods that during some years past have been ranked as tentative. There may have been, in a few instances, neglect on the part of the referee to conduct further investigations or make necessary recommendations. Attention is first directed to paragraphs under the headings **17—Sour Serum** and **18—Zeiss Refractometer Reading of Copper Serum**. The procedures described under these headings have for many years been tried out in dairy and food laboratories, and from the standpoint of good technique and accuracy no reason exists for their continued ranking as tentative.

The qualitative tests described under the headings **19—Gelatin** and **21—Coloring Matters** are the regular well-known procedures that have been applied during many years past in dairy and food laboratories. No criticisms of these methods have come to the attention of the referee, and no modifications have been proposed.

The method for determination of *Sucrose in Sweetened Condensed Milk*, described in paragraphs **38** and **39**, is in its present form considered to be reliable and is, in fact, practically the only procedure available for the purpose to which it is applied. No alternative method has ever been proposed, and no criticisms of the present method have appeared in the literature during the past four years.

Under the section headed *Examination of Fat*, **50**, the qualitative test for *Coloring Matters* appears in the form in which it has been described during many years. This test is, in fact, the original well tried out procedure published originally by Doolittle<sup>1</sup>. This test constitutes the regular method for examination of coloring matter in butter and its substitutes, and it has been practiced in dairy and food laboratories fully 20 years.

The two qualitative tests that now appear under the heading *Renovated Butter and Oleomargarine*, **51** and **52**, may be questioned chiefly on the ground that they do not constitute analytical procedures of primary importance, although both have doubtless been applied extensively for many years. Regarded from a certain viewpoint these tests may be dropped from the chapter without serious inconvenience, but in view of the fact that they appear to serve a certain useful purpose it is the judgment of the referee that they should remain in their present form as tentative.

Under the section headed *CHEESE, Moisture*, **54**, is described a procedure that has recently been subjected to investigation by one of the associate referees and that will be discussed in a report to be submitted

<sup>1</sup> Rpt., Dairy and Food Com., Michigan, 1903, p. 182.

presently, followed by a recommendation favoring the adoption of an alternative tentative method. It will also be recommended that a comparative study of these two methods be conducted as a part of the collaborative work for the ensuing year.

The paragraph headed **FAT, 60**, *The Schmidt-Bondzynski Method, Modified*, still appears, in the experience of the referee and in the judgment of a number of collaborators, reasonably satisfactory and capable of yielding good results. It does not seem, however, that experience with this method will justify any recommendation except that it remain in its present form as tentative.

No justification is discoverable for the appearance under **61** of the so-called *Babcock Method*. It will be remembered that all of the known modified Babcock procedures, as applied to various dairy products other than milk and cream, were subjected to thorough collaborative study under the direction of the present referee during the years 1914-1917, with the result that none of them could be recommended for adoption. It is apparent that paragraph **61** is an inheritance from early editions of the official methods of analysis and may have been retained in the present volume because it was not realized by former referees that the association had acted adversely on all modified Babcock procedures. Furthermore, it is the opinion of individuals consulted, as well as of the referee and his associates, that any modified Babcock method applied to cheese is unreliable. It may also be noted that the method in its present form implies that it may be serviceable for any variety of cheese, and also that it is directed to weigh out about 6 grams of sample and make a calculation that amounts practically to multiplying the fat reading by three.

Under the heading **EXAMINATION OF FAT, 62 (a) and (b)**, appear directions for the preparation of sample to be subjected to physical and chemical tests as directed under Chapter *XXVII*. These procedures are, in the opinion of the referee, quite satisfactory. In fact, both have been applied in dairy and food laboratories to a considerable extent during many years. No criticisms or amendments have ever been suggested.

Under the section headed **ICE CREAM (PLAIN)**, appear the two present tentative methods **64** and **65**. It is believed that the directions for preparation of sample, when properly revised, are entirely satisfactory to those that have given this paragraph a careful study. Regarding paragraph **65**, *Fat, Roese-Gottlieb Method*, it hardly requires any discussion to emphasize the fact that this procedure as applied to plain ice cream is the only reliable one available at the present time. It will be recalled that the standard Roese-Gottlieb method was adopted as official several years ago, also that results of collaborative studies under the

direction of the present referee led to the conclusion that the method was reliable for plain ice cream.

The procedure given under 66 was adopted as a tentative method a number of years ago. Inquiries regarding the application of the method in dairy and food laboratories lead to the conclusion that nowhere is it put to practical use. Therefore, so far as relates to the purposes for which A. O. A. C. methods are formulated, it seems desirable that this procedure for determining fat in ice cream be omitted.

#### RECOMMENDATIONS<sup>1</sup>.

In concluding this report, the following recommendations are made:

(1) That the Babcock method submitted by the referee at the meeting in 1923<sup>2</sup>, be adopted as an official method. (Final action.)

(2) That the following methods indicated by chapter and paragraph headings as they appear in the present *Book of Methods* and also indicated under headings as they are to appear in the forthcoming volume be adopted as official:

(a) MILK—1, Collection of sample, and 2, Preparation of sample (in revised *Book of Methods*);

(b) The serum examination methods under XXI, *Dairy Products*, 17 and 18 (21—II, (a) and (b), 22—III, revised methods);

(c) The methods given under XXI, *Dairy Products*, 19 and 21, and referred to under 28 (26, 28, 39, and 41, revised methods);

(d) CREAM—29, Collection of sample, and 30, Preparation of sample (revised methods);

(e) The method for *Sucrose* in *Sweetened Condensed Milk*, XXI, *Dairy Products*, 38 and 39 (59 and 60, revised methods);

(f) The test given under XXI, *Dairy Products*, 50, (73 and 75, revised methods);

(g) The methods given under XXI, *Dairy Products*, 62 (a) and (b) (87 (a) and (b), revised methods);

(h) The method given under XXI, *Dairy Products*, 64 and 65, (88 and 89, revised methods).

(3) That the following be dropped:

The methods appearing under XXI, *Dairy Products*, 61 and 66.

(4) That the following be adopted as tentative:

(a) The method for Specific Gravity—3 (revised methods);

(b) Acidity—4 (revised methods);

(c) MALTED MILK (revised methods)—61, Preparation of sample; 62, Moisture; 63, Protein; 64, Ash; 65, Fat.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 270.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 9.

## REPORT ON MOISTURE IN CHEESE.

By LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station, St. Louis, Mo.), *Associate Referee*.

During the past year two methods for the determination of moisture in cheese were studied. One was the present tentative A. O. A. C. method<sup>1</sup> consisting essentially of drying the product to constant weight at atmospheric pressure in open dishes with asbestos as the absorbent material. The other, a proposed vacuum method, in which the sample was dried to constant weight in loosely covered dishes without any absorbent material.

The American Brick and the Sicilian Caciocavallo cheeses were used, the former as a representative type of a cheese comparatively high in moisture, and the latter a type of fairly low moisture content. Each cheese was grated, allowed to stand at 3°-5°C. for 3 days in a large, closed desiccator without any desiccating agent, then thoroughly mixed and transferred to several pint Mason jars.

## PROPOSED METHOD.

*Apparatus*.—Aluminum dish, flat bottom with slip-in lid; diameter 58 mm.; height 17 mm.; described in the E. & A. Catalog AA of Chemical and Metallurgical Laboratory Supplies, 1920 edition, under No. 2605.

*Method*.—Transfer 2-3 grams of the sample to a previously tared aluminum dish, cover tightly, and reweigh. Dry the loosely covered dish containing the sample to constant weight in vacuo at a pressure not to exceed 4 inches (100 mm.) of mercury at the temperature of boiling water. During the drying admit to the oven a slow current of air, about 2 bubbles per second, dried by bubbling through concentrated sulfuric acid. The metal dish must be placed in direct contact with the metal shelf of the oven. Release the vacuum carefully. Press the cover tightly into the dish; then remove dish from oven, cool, and weigh. Calculate the loss in weight as moisture.

(NOTE: It is suggested that different weighed portions be dried for 3, 4, and 5 hour intervals, as it is believed that one of these intervals will give the time to obtain constant weight.)

The results of this study were contributed by the following collaborators, to whom the writer at this time desires to express his thanks: Samuel Alfend, U. S. Food and Drug Inspection Station, St. Louis, Mo.; O. L. Everson, Bureau of Chemistry, Washington, D. C.; Louis Katz, U. S. Food and Drug Inspection Station, New York City, N. Y.; J. T. Keister, Bureau of Chemistry, Washington, D. C.; O. S. Keener, U. S. Food and Drug Inspection Station, St. Louis, Mo.; and C. A. Roach, U. S. Food and Drug Inspection Station, Chicago, Ill. Their results are given in the table.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 278.

*Results of determinations of moisture in cheese.*

ANALYST	AMERICAN BRICK				SICILIAN CACIOCAVALLO			
	Tentative A. O. A. C. method	Proposed vacuum method			Tentative A. O. A. C. method	Proposed vacuum method		
		3 hrs.	4 hrs.	5 hrs.		3 hrs.	4 hrs.	5 hrs.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Alfend .....	40.10 39.95	40.45 41.35	41.20 .....	40.28 40.29	29.01 28.76	28.51 28.62	28.50 28.58	28.48 28.80
Everson .....	41.18 ..... 41.03 41.05 41.65 41.45 41.91 41.08 .....	41.09 41.20 40.87 40.42 ..... 41.09 41.60 40.92	41.05 40.42 41.21 41.57 41.72 ..... 41.09 ..... 41.23	41.06 41.56 ..... ..... 41.36 41.28 ..... .....	28.70 ..... 29.40 29.18 29.42 29.04 28.70 28.73 .....	28.74 28.44 ..... 28.90 ..... 28.39 28.55 28.44 28.65	28.50 28.68 28.70 28.92 28.80 28.99 28.79 28.70 28.55	28.48 28.78 28.35 28.50 ..... 28.60 ..... 28.33 28.30
Katz .....	41.37 41.50	41.14 .....	41.29 .....	41.58 .....	28.64 28.54	28.42 .....	28.55 .....	28.71 .....
Keister .....	41.61* 41.76 40.68† 40.70	42.05 41.92 41.68 41.65	42.07 42.17 41.82 .....	42.08 42.12 41.79 41.68	28.36 28.44 ..... .....	28.48 28.41 ..... .....	28.37 28.34 ..... .....	28.61 28.59 ..... .....
Keener .....	41.08 41.07	41.22 40.95	41.12 41.25	41.13 41.00	27.93 28.03	28.78 28.71	28.41 28.69	28.51 28.61
Roach .....	40.83 40.76	40.53 40.60	40.74 40.86	40.62 40.78	27.66 27.78	28.03 28.09	28.32 28.20	28.20 28.17
Average	41.09	41.15	41.30	41.24	28.61	28.51	28.61	28.54
Maximum	41.91	42.05	42.17	42.12	29.42	28.90	28.99	29.13
Minimum	39.95	40.42	40.42	40.28	27.66	28.03	28.20	28.17
Variation	1.96	1.63	1.75	1.84	1.76	0.87	0.79	0.96

\* First sample

† Second sample.

## COMMENTS BY COLLABORATORS.

## A. O. A. C. METHOD.

*Alfend*.—From 1–5 mg. loss in weight during weighing out of sample when open platinum dish was used. Difficult to mix Brick cheese well with asbestos.

*Everson*.—Sand was used instead of asbestos. Four or five weighings were necessary in most cases. In case of Caciocavallo No. 2 (29.40) eight weighings were made. A Freas electric oven was used. Temperature kept as near 100°C. as possible. Sample cooled in desiccator over sulfuric acid.

*Katz*.—Drying done with asbestos in platinum dishes. Sample used, about 4.0 grams. Temperature of oven, 100°C. First drying, 2 hours; second, 1½ hours; third, 1 hour. American Brick registered slight gain in weight (1 mg.) on third drying; Sicilian Caciocavallo, a loss of 5 mg., equivalent to about 0.1 per cent. Oven used, Central Scientific Company's electric oven with de Khotinsky constant temperature appliance.

*Keister*.—Asbestos was used, and the sample was dried at 100°C. in a water-jacketed

oven, the size dishes specified being used. The sample was then cooled in a desiccator over sulfuric acid. Only three weighings were necessary for both samples.

*Keener*.—Maximum loss third weighing for Caciocavallo, fourth weighing for Brick.

*Roach*.—The method calls for drying in oven at 100°C. and weighing at 1–1½ hour intervals until weight becomes constant. The electric oven was used in this work with a temperature of 100°–104°C. Maximum loss in weight was obtained in the second weighing. The third weighing showed a slight gain.

#### PROPOSED VACUUM METHOD.

*Alfend*.—Maximum values in 4 hours drying. Sample of Brick cheese tends to foam over with loss of fat, unless quite small sample is used. Method is more convenient than A. O. A. C. No dry crust forms during drying.

*Everson*.—The temperature was kept at 97°–100°C. The pressure varied from 97–100 mm. mercury. Freas electric oven was used. Dishes were as specified in method.

*Katz*.—Sample used, about 2.5 grams. Drying done at boiling water temperature and 28 inch vacuum in water-jacketed vacuum oven. Aluminum dishes used of dimensions given in method. American Brick foamed up, pushing the cover slightly, but apparently no loss of material sustained. Cooling for 20 minutes in calcium chloride desiccator allowed in all cases.

*Keister*.—The vacuum varied, being 25 inches for both samples as first received and 27 inches on the second portion of the Brick sample. It was first thought that some of the wild figures obtained on the Brick cheese were due entirely to the non-uniformity of the sample, but later experience on the second portion leads to the conclusion that slight losses of fat while drying in the oven in some cases contributed to the errors, as several determinations were lost due to loss of fat by creeping over the sides of the dish. This condition was due in part to the size of sample taken (in some cases over 3 grams) and possibly in part to the age and condition of the sample. Therefore, cheese of this character should be examined when fresh and not over 2 grams used in a dish of the size specified in this work.

#### DISCUSSION OF RESULTS.

In view of the high moisture content of cheese, the inherent difficulty of preparing a uniform sample, and the almost universal lack of duplicating exact conditions during drying, the results of the different collaborators are considered fairly uniform when compared as a whole. Until the last two difficulties can be overcome, a fair comparison of methods for the determination of moisture can be made only by considering separately the work of each analyst. Four of the analysts reported higher moisture results by the proposed vacuum method on both samples of cheese than by the tentative A. O. A. C. method; one obtained higher values by the vacuum method on the Brick cheese, but lower results on the Caciocavallo sample. The results of Everson would appear to confirm the difficulties that the referee experienced sometime ago when using the Freas electric oven<sup>1</sup>.

In the proposed vacuum method, particularly in the case of the Brick cheese, several analysts noted a tendency for the fat to creep over the sides of the dish. This trouble may be avoided by using a smaller sam-

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5 498



ple or by partially drying the sample on a steam bath before subjecting it to the high vacuum.

On the average, the highest moisture values for the vacuum method were obtained on 4 hours drying, although in some instances the 3 hour period was sufficient, and in others drying for 5 hours was necessary.

In the A. O. A. C. method, Alfend reported that as much as 0.20 per cent of moisture may be lost when weighing a sample of high-moisture cheese. This error is obviated in the proposed method.

Though some analysts have found two weighings sufficient in the A. O. A. C. method, others have found it necessary to make as many as seven or eight. In the vacuum method, 5 hours drying is always sufficient.

#### CONCLUSION.

It appears that the proposed vacuum method yields moisture results as uniform as those obtained by the present tentative A. O. A. C. method, and consistently higher. The method requires no auxiliary substance, such as asbestos or sand, and is far more convenient in regard to time and effort.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the vacuum method for the determination of moisture in cheese be adopted as tentative.

(2) That the comparative study of the present tentative and vacuum methods for the determination of moisture in cheese be subjected to further collaborative work, including a study of the toluene distillation method.

(3) That in view of the difficulties in obtaining uniform samples, each collaborator be requested to make the study on samples prepared by himself.

#### REPORT ON FAT IN DRIED MILK<sup>2</sup>.

By J. T. KEISTER (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

In accordance with the recommendation adopted at the meeting held in November, 1923, relating to methods for determining fat in dried milk, the Jephcott modification of the Werner-Schmidt method was subjected to study. Before the work had progressed very far, the writer decided that the method had an objectionable feature, namely, that the quantity of heat to which the sample is subjected results in the charring of the lactose, a portion of which is dissolved in the ethers and carried over with the fat. The quantity of ether-soluble non-fat material ap-

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 271.

<sup>2</sup> Presented by Julius Hortvet.

pears to vary from about 1.5–5 mg., which must be determined in each case and a proper correction applied. It may be stated that in samples of very high fat content (like No. 10 in the table) the soluble non-fat material is very slight—less than 1 mg. The Jephcott procedure is as follows:

Weigh 1 gram of sample into a hard-glass boiling tube 8 inches by 1 inch. Add 8 cc. of water and 2 drops of ammonia (sp. gr. 0.880). Gently boil the mixture until all lumps are dissolved. Add 10 cc. of concentrated hydrochloric acid (sp. gr. 1.16) and heat the tube in a Bunsen flame with constant gentle agitation. Note the time the boiling begins and continue boiling for 3 minutes. Cool the tube somewhat; add 50–70 cc. of ether, and, after standing for a short time, blow off the ethereal layer by means of the usual wash bottle fitting. On this first extraction, the ether is not shaken with the acid mixture. The major portion of the fat is on the surface of the acid liquor and readily dissolves in the ether. A second portion of 50–70 cc. ether is added and the tube well shaken. Allow to stand for at least 30 minutes and blow off into the same flask. The third extraction may be made if necessary, depending on the fat content of the sample and also how closely the ether has been blown off. Recover the ether by distillation and dry the contents of the flask at 102°C. for 1 hour. Dissolve the fat in petroleum ether, using three rinsings of about 10 cc. each. Dry the flask again and cool. Weigh. The difference in weight is taken as fat.

In carrying out the method a small beaker was used instead of the tube for boiling the sample. The extraction was made in a Röhrig tube and the drying done at 100°C. to constant weight instead of at 102°C. for 1 hour.

On account of the unsatisfactory results that were obtained, it was decided to attempt an improvement on the method by resorting to a procedure, with slight changes, that had been tried out some years ago on both malted milk and dried milk. The results obtained by this method are given in Column 2 of the table. The procedure is as follows:

Weigh about 1 gram of well mixed sample into a small beaker. Add about 1 cc. of water. Mix thoroughly with a glass rod to a thick, smooth liquid. Add 10 cc. of concentrated hydrochloric acid. Heat in a water bath at 80°C. for 10 minutes. Cool, transfer to a Röhrig tube, and add about 10 cc. of 95 per cent alcohol. Mix, and proceed with the extraction as in the official Roese-Gottlieb method for milk. Dry to constant weight at 100°C. Dissolve the fat in petroleum ether. Dry the flask, cool, and weigh. Correct results for any residue that may be present.

This method usually yields a very small quantity (less than 1 mg.) of insoluble material and also a fat slightly darker than that by the Roese-Gottlieb method but not nearly so dark as the fat obtained by the Jephcott process. It should be stated that in most samples of skimmed milk powder results by the Roese-Gottlieb method show about 0.5 mg. of insoluble material. Therefore in all three methods included in this report, the dry fat was dissolved in petroleum ether, and the flask was dried and then reweighed for insoluble residue. It was only after some experience and trouble with emulsion formation in the acid extraction

method that 10 cc. of alcohol was substituted for the same volume of water, which change showed no variation in the results and was a distinct advantage because no further trouble with emulsion formation was experienced. The results, therefore, seem to indicate that the alcohol does not play any part in the fat extraction in this procedure but merely serves to avoid the formation of emulsions.

By eliminating sample No. 2, it is found that 10 out of 13 samples run slightly high by the acid treatment as compared with results by the Roese-Gottlieb method, the average difference being  $+0.08$  per cent. The three samples that run low by the acid method average  $-0.08$  per cent difference, which would give a general average difference of only about  $+0.06$  per cent by the acid treatment. No explanation can be given as to why sample No. 2 yielded a high result by the Roese-Gottlieb method. In the case of sample No. 7, it should be stated that this was a very old and rancid sample and that the fat by the Roese-Gottlieb method ran about 2 per cent low, indicating that only reasonably fresh samples should be examined for fat content by the ammonia treatment.

Aside from the objectionable point already mentioned, it is noted that the results by the Jephcott treatment are high with two exceptions (Nos. 14 and 15), varying from 0.02–1.11 per cent, and also that the results were not so consistent as those obtained by the other two methods. Although the results indicate that the acid treatment extracts slightly more fat than the Roese-Gottlieb procedure, the data available are not sufficient to recommend this modification. Considering the results submitted in the table, as well as the results reported a year ago, the Roese-Gottlieb method appears to be sufficiently accurate to be adopted as tentative for dry milk, in the following form:

Weigh out about 1 gram of well mixed sample into a small lipped beaker. Add about 1 cc. of water. Mix well with a glass rod to form a thick liquid free from lumps. Add 9 cc. more of water and 1 cc. of ammonia. Warm on the steam bath. Transfer to a Röhrig tube or similar apparatus. Cool. Add 10 cc. of 95 per cent alcohol. Mix. Add 25 cc. of ethyl ether and proceed with the extraction as in the official Roese-Gottlieb method for milk. Dissolve the dried fat in petroleum ether and determine the quantity of any insoluble residue that may be present. In case of whole milk and cream powders, make a third extraction, using a mixture of 15 cc. of each ether.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the Roese-Gottlieb method, as described in the preceding paragraph, be adopted as a tentative method for dried milk.

(2) That the method described by Holm<sup>2</sup> and published in the proceedings of the meeting held in 1921 be subjected to collaborative study.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 271.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1921, 5: 509.

*Fat in milk powder.*

(All results calculated to a dry basis)

SAMPLE NO.	JEPHCOTT PROCEDURE		HYDROCHLORIC ACID METHOD BY HEATING TO 80°C. FOR 10 MINUTES		OFFICIAL ROESE-GOTTLIEB METHOD		DIFFERENCE IN RESULTS SHOWN IN COLUMNS 2 AND 3
	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>	<i>average per cent</i>	<i>per cent</i>
1	28.198 27.57	27.88	27.35 27.29	27.32	27.242 27.286	27.26	+ 0.06*
2	29.55 30.32	29.93	30.05 ...	30.05	30.78 30.628	30.70	- 0.65
3	28.19 ....	28.19	27.03 27.14	27.085	27.025 ....	27.025	+ 0.06
4	1.48 1.59	1.53	1.35 ....	1.35	1.397 1.325	1.36	- 0.01
5	0.725 0.97	0.847	0.886 0.846	0.866	0.992 1.024	1.008	- 0.14
6	0.978 0.983	0.98	0.936 ....	0.936	0.877 0.880	0.878	+ 0.058
7	29.07 30.22	29.64	28.55 28.59	28.57	....	....	.....
8	1.01	1.01	0.964 0.977	0.97	1.026 1.111	1.068	- 0.09
9	1.09 1.36	1.22	1.02 1.023	1.02	1.025 0.998	1.01	+ 0.01
10	55.13 54.67	54.90	54.45 54.51	54.48	54.228 54.274	54.25	+ 0.23
11	1.177 1.133	1.15	0.453 0.666	0.549	0.507 0.483	0.495	+ 0.054
12	1.90 1.90	1.90	1.51 1.546	1.53	1.47 1.43	1.45	+ 0.08
13	20.05 19.84	19.94	19.705 19.818	19.76	19.69 19.73	19.71	+ 0.05
14	1.00 .	1.00	1.19 0.998	1.09	0.97 ....	0.97	+ 0.12
15	11.60 ....	....	12.23 12.46	12.34	12.40 12.52	12.46	+ 0.12

\* + sign = high by acid method; - sign = low by acid method.

## REPORT ON FATS AND OILS.

By GEORGE S. JAMIESON (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

During the past year the referee, with the assistance of his collaborators, has continued the study of the determination of unsaponifiable matter in fats and oils as recommended. A comparative study of the association's official method with the André-Cook method for the determination of acetyl value was undertaken.

## DETERMINATION OF UNSAPONIFIABLE MATTER.

The three methods studied were the following: F. A. C. (Fat Analysis Committee of the Division of Industrial Chemists and Engineers, American Chemical Society<sup>1</sup>, a modification of the official A. O. A. C. procedure<sup>2</sup>, and the new Kerr-Sorber method<sup>3</sup>.

*Modified A. O. A. C. Method.*

Saponify 5 grams of the sample with 5 cc. of 50 per cent aqueous potassium hydroxide and 30 cc. of 95 per cent ethyl alcohol. Remove most of the alcohol by evaporation. Dissolve the residue in 75 cc. of water and transfer all the solution to a separatory funnel, keeping the final volume below 120 cc. Rinse the saponification flask with about 60 cc. of ether, which is added to the cool aqueous solution in the separatory funnel. Shake thoroughly and let stand until the two layers have *completely separated*. (Two cc. of alcohol may be added to facilitate the breaking of the emulsion, which is usually very persistent.) Withdraw the aqueous solution into another separatory funnel and extract again with 50 cc. of ether. Make in all 5 ether extractions and collect them in a 500 cc. separatory funnel. Wash with 25 cc. of water by rotating the funnel gently in order to avoid the formation of a troublesome emulsion. Allow the solutions to stand 10 minutes, then withdraw the aqueous layer as completely as possible. Repeat the washing with 25 cc. portions of water until no test is given with phenolphthalein. Usually three washings are sufficient. Distil the ether from a weighed flask and dry the residue for an hour, preferably in a vacuum oven heated to 100°C.; then cool and weigh. Repeat the drying until a constant weight is obtained. Test the purity of the residue in every case. Add 10 cc. of anhydrous alcohol, free ether, or dry petroleum ether and, if necessary, warm slightly. In the absence of soap, the entire residue will dissolve. In case an insoluble residue remains, remove the unsaponifiable matter completely from the flask by rinsing with small portions of anhydrous solvent; again dry the flask containing the soap attached to the bottom; weigh; and deduct from the first weight.

*Kerr-Sorber Method.*

Weigh 5 grams of the sample into a 200 cc. Erlenmeyer flask and add 15 cc. of 95 per cent ethyl alcohol. To another 15 cc. portion of alcohol in a flask, add 3 cc. of an aqueous solution of potassium hydroxide (100 grams of alkali in 100 cc. of water). Heat both solutions to boiling. Pour the alkali solution into the flask containing the sample

<sup>1</sup> *J. Ind. Eng. Chem.*, 1919, 11: 161.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 295.

<sup>3</sup> *Cotton Oil Press*, 1924, 7: 40.

and mix if necessary by gently rotating the flask. Boil gently for 10 minutes; then cool to room temperature. Add 50 cc. of ether, mix, and transfer to a separatory funnel. Rinse the flask with two successive 50 cc. portions of ether, adding them to the separatory funnel, and mix by gently rotating the funnel. Add 150 cc. of water, pouring it into the separatory funnel in a slow steady stream. Rotate the funnel gently to secure better contact of the solutions, *but do not shake*. Shaking at this stage results in the formation of a stubborn emulsion. Separation takes place at once and is sharp. Draw off the soap solution and wash the ether solution with two successive 100 cc. portions of water, still without shaking. Continue the washing until the last portion is free from alkali and soap, as shown by testing with phenolphthalein indicator. Transfer ether to a weighed beaker or a flask, remove the ether, and dry to constant weight (preferably according to the F. A. C. procedure).

*The F. A. C. Method<sup>1</sup>.*

For the study of the determination of unsaponifiable matter by the three methods, five representative samples were selected. However, the fifth sample (E), which was much smaller than the others, was not available to all the collaborators. Sample A was a refined coconut oil; B, a refined cottonseed oil; C, a yellow grease; D, a tallow; and E, a brown grease.

The results of the analyses are given in Table 1.

Examination of the results reported for each sample show that regardless of the method employed, a number of them are considerably above or below the general range given. In the case of the F. A. C. results it is possible that some of the higher figures are due to making more than the customary five petroleum ether extractions. Although more than five extractions are permitted, such cases should have been reported so that fact could be indicated in the table. For example, the referee made seven extractions in the analysis of Sample E and obtained 3.79 and 3.82 per cent, while five extractions gave 3.41 and 3.42 per cent, as stated in the table.

Several of the collaborators included both the uncorrected and corrected percentages obtained. The corrected figures, which they believed were the more accurate, are the ones given in Table 1, unless otherwise indicated.

It should be definitely understood by those interested that it is not the intention of the referee to have the modified A. O. A. C. procedure adopted by the association on account of the troublesome emulsions encountered, which make it unduly long and tedious. Since it is believed that this procedure is capable of giving results of a high order of accuracy, when properly conducted, it was introduced into the past year's work to furnish data by which the accuracy of the other two methods could be compared.

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 85.

TABLE 1.  
*Unaponifiable matter.*

COLLABORATORS	MODIFIED A. O. A. C. METHOD— 5 ETHER EXTRACTIONS					KERN-SORBER METHOD					F. A. C. METHOD				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
R. W. Bailey 95 Front St., New York, N. Y.	0.40	0.78	1.68	2.38	....	0.20	0.60	1.38	2.16	....	0.24	0.68	1.28	2.02	....
Victor Conquest Proctor & Gamble, Ivorydale, Ohio	0.28 0.38	0.80 0.90	1.60 1.27	2.36 2.24	4.42 4.49	0.28 0.34	0.68 0.67	1.72 1.65	2.22 2.30	3.73 3.79	0.25 0.33	0.83 0.75	1.19 1.07	1.94 1.85	4.04* 4.06*
A. Edeler Savannah, Ga.	0.34	0.73	1.42	2.04	....	0.25	0.77	1.70	2.23	....	0.45	0.61	1.07	1.81	....
G. S. Jamieson	0.27 0.24 0.24	0.75 0.77	1.44 1.46 1.50	2.26 2.30 2.30	3.92 3.94	0.27 0.28	0.83 0.83	1.57 1.61	2.22 2.30	3.92 3.99	0.20 0.22	0.60 0.62	1.08 1.13	1.91 1.93	3.42 3.41
C. A. Knuth Proctor & Gamble, Ivorydale, Ohio	0.35	0.83	1.94	2.73	....	0.39	0.64	1.44	2.13	....	0.41	0.73	1.22	1.81	....
J. R. Powell Armour Glue Works, Chicago, Ill.	0.48	0.93	1.18	1.94	....	0.45	0.67	1.16	2.11	....	0.47*	0.83*	1.18*	1.61	3.18
W. D. Richardson Swift & Co., Chicago, Ill.	0.33	0.74	1.16	1.87	3.57	0.20	0.53	1.17	1.76	3.28	0.29*	0.71*	1.02*	1.52*	3.30*
H. P. Strack Savannah, Ga.	0.27 0.24	0.85 0.95	1.49 1.23	2.14 2.37	3.91 3.97	0.28 0.31	0.85 0.84	1.44 1.30	2.22 2.37	3.73 3.61	0.31 0.35	0.67 0.82	1.07 1.00	1.90 1.53	3.44 3.48
J. T. Parsons H. J. Heintz Co., Pittsburgh, Pa.	0.27 0.26	.... ....	.... ....	.... ....	4.68 4.68	0.32 0.34	0.83 0.91	1.65 1.53	2.03 1.94	3.65 3.48	.... ....	.... ....	.... ....	.... ....	.... ....

The figures reported by Powell and Richardson, with the exception of those noted otherwise, have been corrected for free fatty acids as oleic acid determined by titrating with 0.1 *N* alkali the solution of the unsaponifiable residues dissolved in warm neutralized alcohol. Powell comments in part as follows: "You will note that in general the figures obtained by the A. O. A. C. and Kerr-Sorber methods are considerably higher than those of the F. A. C. We believe that this is accounted for by the fact that in the two former methods we are dealing with solutions of soap in water, extracting the unsaponifiable with ethyl ether, both of which operations have a tendency to hydrolyze the soap to a considerable extent. With the F. A. C. method, on the other hand, we are dealing with a 50 per cent alcoholic soap solution in which hydrolysis occurs only to a limited extent. You will note that we have taken the liberty of correcting the figures for this fatty acid content".

When these comments were received, no time remained prior to the annual meeting of the association for further study of the Kerr-Sorber method by the referee, but this matter will be further investigated as soon as possible.

The lower results obtained in the F. A. C. method may be due to the fact that certain compounds characteristic of unsaponifiable matter of fats and oils, for example cholesterol and phytosterols, have a very considerable solubility in alcoholic soap solutions. Therefore the lower results obtained by the F. A. C. method are explainable in part on the basis of incomplete extraction. (The referee has shown previously that seven petroleum ether extractions in the case of Sample E extracted 4 per cent more than was obtained by five extractions. This further extraction of unsaponifiable matter in weighable quantities by making six to nine extractions has been frequently observed by the referee. In the 1923 report, it was stated that more than five extractions (F. A. C.) appeared to be necessary for substances containing over 1 per cent of unsaponifiable matter.) This hypothesis is consistent with the fact that the differences between the methods are larger on the samples that show the highest unsaponifiable matter and least on those that have the least unsaponifiable matter.

In the case of the Kerr-Sorber method the chances of hydrolysis would appear to be much reduced as compared with the modified A. O. A. C. procedure, in which the saponified fat is dissolved in a considerable volume of water, from which the unsaponifiable matter is subsequently extracted. With the latter method the correction due to hydrolysis of the soap should be the larger. However, this apparently is not borne out by the data at hand. Referring again to the 1923 report, two analyses will be found in which the residues of unsaponifiable matter, after being weighed, were heated with alcoholic potash and again extracted according to the modified A. O. A. C. method, with the result that the percent-



age of the "purified" unsaponifiable matter differed only by 0.02 per cent from the figures first obtained. In the case of these two analyses it appears that no measurable quantities of free fatty acids were weighed together with the unsaponifiable. The recent well-known researches of J. W. McBain have shown that the actual amount of hydrolysis of the soap in very dilute aqueous solutions is small. The hydrolysis of soaps in solution is even less in the presence of free alkali, alcohol, and glycerin.

If the following additional results by the F. A. C. method, which were reported by one collaborator, are representative, it would indicate that the "hydrolysis" of the soap with the formation of some free fatty acids applies also to this method.

METHOD	SAMPLE D		SAMPLE E	
	Uncorrected	Corrected	Uncorrected	Corrected
F. A. C.	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	1.85	1.73	3.44	3.26
	1.95	1.77	3.68	3.47

In order to prove definitely that fatty acids were actually present in the unsaponifiable matter obtained by any or all of these methods, the referee believes that the fatty acids should be actually isolated and weighed.

In view of the agreement shown by the majority of results reported for the Kerr-Sorber method with those obtained by the modified A. O. A. C. method, it is proposed that the Kerr-Sorber method be made official and that the first reading of this recommendation be made. If subsequent study should prove that it is not the method best adapted to give accurate results, naturally another method would be recommended. It is recommended that further work be done on the determination of unsaponifiable matter.

#### ACETYL VALUE.

##### *André-Cook Method.*

Boil the oil or fat with an equal volume of acetic anhydride for 2 hours, pour the mixture into a large beaker containing 500 cc. of water, and boil for 30 minutes. To prevent lumping, pass a slow current of carbon dioxide into the liquid through a finely drawn-out tube reaching nearly to the bottom. Allow the mixture to separate into two layers. Siphon off the water and boil the oily layer with fresh water until it is no longer acid to litmus paper. Separate the acetylated fat from the water and filter through paper in a drying oven. Determine the saponification values of the oil before and after acetylation. Calculate the acetyl value by the following formula:

$$A = \frac{S' - S}{1 - 0.00075S}, \text{ in which}$$

$A$  is acetyl value,  $S$  is saponification value before acetylation, and  $S'$  is saponification value after acetylation<sup>1</sup>.

<sup>1</sup> *Compt. rend.*, 1921, 172: 984; *Bull. Soc. Chim.*, 1921, 29: 745; *J. Am. Chem. Soc.*, 1922, 44: 392.

*Official A. O. A. C. Acetyl Value Method<sup>1</sup>.*

For the study of the determination of acetyl value by the two methods, four samples of oils were distributed to the collaborators. Sample 1 was a mustard seed oil containing about 20 per cent of castor oil; Sample 2, a crude Chinese mustard seed oil; Sample 3, a refined corn oil; and Sample 4, a refined cottonseed oil.

TABLE 2.  
*Collaborative results on the determination of acetyl value.*

COLLABORATORS	A. O. A. C.—FILTRATION METHOD				ANDRÉ-COOK METHOD			
	1	2	3	4	1	2	3	4
A. Edeler . . . . .	31.9	8.8	8.0	9.2	29.3	4.8	5.5	6.1
C. A. Knuth . . . . .	43.8	17.3	16.5	17.2	39.3	9.7	10.0	10.8
H. P. Strack . . . . .	48.32 37.08	16.74 10.20	10.09 10.20	11.43 10.19	36.39 35.54	7.75 7.21	9.24 9.11	10.90 11.08
W. F. Baughman . . . Bureau of Chemistry Washington, D. C.	39.3 40.3	14.0 14.6	10.9 6.5	11.0 14.0	39.3 39.2	10.1 11.4	7.4 13.3	14.0 19.5
W. D. Richardson . . .	54.0 53.9	33.2 32.9	35.2 35.8	40.9 37.2	46.7 46.4	12.2 12.2	13.0 13.82	13.4 14.28
J. R. Powell . . . . .	64.9 61.3	25.6 lost	19.2 19.0	16.6 17.3	55.0 55.0	14.0 14.1	9.8 10.0	9.6 9.6

The figures reported for the analysis by either method show so little agreement among themselves that it is obvious that it will be necessary to continue this investigation this coming year. In so far as the referee can ascertain, the large variations in the results are probably due either to incomplete acetylation or to insufficient purification of the acetylated oil. A number of collaborators stated in their reports that they regretted not having more time in which to repeat the analyses and become better acquainted with the methods.

It is recommended that the work be continued.

The referee takes this opportunity to express his appreciation and to thank those who have collaborated with him during the past year.

RECOMMENDATIONS<sup>2</sup>.

It is recommended—

- (1) That further work be done on the determination of acetyl value.
- (2) That further work be done on the determination of unsaponifiable matter.
- (3) That the Kerr-Sorber method for the determination of unsaponifiable matter be made official.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 293

<sup>2</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8 272.

## REPORT ON BAKING POWDER.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Referee*.

In 1924 the Referee on Baking Powder desired to secure collaborative results from several analysts working with uniform samples trying out the tentative methods as adopted by this association for the determination of lead, carbon dioxide, and neutralizing value of mono-calcium phosphate. The referee, somewhat disappointed at the results received, recommends that further study be made before final action is taken on these methods. The work on fluorine has been conducted by the associate referee, J. K. Morton of the Bureau of Chemistry.

## THE ELECTROLYTIC DETERMINATION OF LEAD.

For this determination, a lead-containing phosphate was prepared by spraying mono-calcium phosphate with a solution of lead nitrate and thoroughly mixing. The prepared phosphate was supposed to contain approximately 225 parts of lead per million, and by analysis it showed 229.4 and 226.8, an average of 228.1 parts per million. This lead-containing phosphate was then made into a baking powder that showed a lead content of 78.84, 79.48, and 77.56, an average of 78.63 parts per million. This baking powder was sent out to collaborators, who reported the results shown in Table 1.

TABLE 1.  
*Determination of lead.*

ANALYST	LEAD	
	Parts per million	Average
L. D. Mathias Victor Chemical Works, Chicago Heights, Ill.	....	59
Milton H. Kemp Calumet Baking Powder Company, Chicago, Ill.	76 72	74
J. L. Howerton Federal Phosphorus Works, Anniston, Ala.	57.6 57.1	57.35
James K. Morton Bureau of Chemistry, Washington, D. C.	78.84 79.48 77.56	78.63
E. W. Thornton R. B. Davis Company, Hoboken, N. J.	75.6 78.2 88.4	80.7

Of the five collaborators, only three obtained results that are reasonably correct. These three are very good indeed and show that reliable results may be obtained by this method if the work is properly carried out as directed. The other two collaborators obtained only about two-thirds of the lead present. Without knowing the actual conditions under which these determinations were made, it is believed that the low results were obtained by failing to use the proper current density, so that the full quantity of lead was not deposited on the electrodes, or if deposited, the conditions were such that it did not remain there until dissolved off.

Since it has been shown that this method is capable of producing accurate results, it is recommended that further collaborative data be obtained from chemists that closely follow the details of the method as outlined.

#### VOLUMETRIC DETERMINATION OF CARBON DIOXIDE.

Two samples of baking powder, one a straight phosphate powder and the other a combination phosphate-S. A. S. powder, were sent to the collaborators for the determination of total and residual carbon dioxide by the volumetric method.

As shown in Table 2 the results of the different determinations agree quite well for any one analyst, but those reported by different analysts show too wide variations. It occurred to the referee that these discrepancies might be due to change in the baking powder itself; accordingly repeat analyses were made by the referee and this was shown to be the case. The referee does not know the dates on which the several analysts made their determinations of carbon dioxide, but the results indicate that they may have been made at different times, and as this powder deteriorated more rapidly than usual the results are not comparable.

Attention is called to the fact that although one of the collaborators used the tentative method of the A. O. A. C., his apparatus differed somewhat in form (but not in principle) from the one used in the tentative method. This collaborator compared his results with the official Knorr method and obtained excellent checks, showing the method to be a reliable one.

In order that collaborative results on carbon dioxide may be of value in checking a method it is essential that all the determinations be made at approximately the same time, and that time should be before the samples have had a chance to deteriorate.

TABLE 2.  
*Determination of carbon dioxide.*

ANALYST	STRAIGHT PHOSPHATE BAKING POWDER						PHOSPHATE-S. A. S. BAKING POWDER					
	Total CO <sub>2</sub>			Residual CO <sub>2</sub>			Total CO <sub>2</sub>			Residual CO <sub>2</sub>		
	No. of Deter-minations	Average	per cent	No. of Deter-minations	Average	per cent	No. of Deter-minations	Average	per cent	No. of Deter-minations	Average	per cent
		per cent			per cent			per cent			per cent	
Milton H. Kemp . . . . .	2*	13.80 13.81	13.805	2	0.28 0.24	0.26	6*	15.04* 15.07 14.88 15.06 15.02 14.84 14.93† 15.17 15.08 14.87 14.85	14.99	3*	0.97 0.99 0.85 0.96 0.83 0.75	0.93 0.84
William G. Warning Provident Chemical Works, St. Louis, Mo J. L. Howerton	3	13.77 13.78	13.775	2	0.26 0.28	0.27	5†	14.98	14.98	3	1.17	
Russel L. Taylor Department of Agriculture, Lansing, Mich	4	13.20 13.10 13.10 13.10	13.13	3	0.50 0.55 0.50	0.52	7	14.67 14.39	14.67	6	0.89	
L. H. Bailey . . . . .	6 (4-5-24)	14.50 14.40 14.30 14.40 14.30 14.20	14.35	6	0.4 0.3 0.3 0.2 0.2 0.4	0.30	3 (4-5-24)	15.0 15.1 15.3	15.13	2	0.7 0.6	0.65
R. E. Phillips R. B. Davis Company, Hoboken, N. J. C. C. Albee R. B. Davis Company, Hoboken, N. J.	3 (9-15-24)	11.30 11.50 11.40	11.40	3	0.30 0.30 0.30	0.30	2 (9-15-21)	13.5 13.5 13.6	13.53		---	1.20
		---	14.10		---	---		---	15.10		---	1.10
		---	14.10		---	---		---	15.05		---	

\* A. O. A. C. tentative method, substituting Harrison apparatus (volumetric).

† Knorr method—official—(gravimetric).

## DETERMINATION OF NEUTRALIZING VALUE.

This determination is one that is of considerable interest to the manufacturers of phosphate as well as of baking powder and self-rising flour. The object of the determination is to find out how much bicarbonate of soda should be used with each lot of mono-calcium phosphate. Different methods have been proposed to show the acid reacting strength of the phosphate; unfortunately, they show different results. The correct titration of a phosphate is a difficult matter, owing to the various reaction products formed under certain conditions and the difficulty of obtaining a sharp end point with an indicator.

TABLE 3.  
*Determination of neutralizing value.*

ANALYST	NEUTRAL- IZING VALUE	AVERAGE	ANALYST	NEUTRAL- IZING VALUE	AVERAGE
F. B. Carpenter Virginia-Carolina Chemical Co. Richmond, Va.	81.0 81.2 80.8	81.0	W. R. Collins Royal Baking Powder Co., Brooklyn, N. Y.	82.20 81.66	81.93
A. H. Allen Virginia-Carolina Chemical Co. Richmond, Va.	81.0 81.0 81.0 81.2 81.2 81.2 81.4 81.6	81.2	H. E. Hintz Royal Baking Powder Co., Brooklyn, N. Y.	81.84 81.96	81.90
J. L. Howerton	. .	84.2	Grace Vincent Royal Baking Powder Co., Brooklyn, N. Y.	82.0 82.1 82.0 82.0	82.0
F. A. Barker Federal Phosphorus Works, Anniston, Ala.	. . .	84.4	E. H. Wright Royal Baking Powder Co., Brooklyn, N. Y.	81.7 81.8 81.7 81.7	81.7
W. C. Luckow American Institute of Baking, Chicago, Ill.	80.60 80.96 81.04 80.60 80.10 80.20 80.44 81.16 80.76 81.40 80.40 81.20	80.74	A. A. McDonald Victor Chemical Works Chicago, Ill.	80.8 81.0	80.9
William G. Warning	. . . .	81.6	A. Awotin Victor Chemical Works Chicago, Ill.	81.4 81.0	81.2
E. W. Thornton	80.-85.		W. R. Stanley Victor Chemical Works Chicago, Ill.	81.2 81.4	81.3
			L. H. Bailey	80.8 81.0 80.6 80.8	80.8
			C. C. Albee	79.0 79.6	79.3
			R. E. Phillips	79.4 79.8	79.6

As shown in Table 3 seventeen collaborators reported results by the tentative method, and with two exceptions the agreement is very good. The two analysts that obtained higher results than the others may have carried their titrations to a point where they obtained a decided color with phenolphthalein instead of stopping with the first appearance of color. One of the collaborators proposed an indirect titration method as a substitute for the tentative method of this association. This proposed method gives a higher neutralizing value to the phosphate than the tentative method. The referee made up experimental baking powders by assuming the neutralizing value to be that obtained by the proposed method and that by the tentative method. Both powders were alkaline, and both contained residual carbon dioxide, the proposed method showing a greater quantity than the tentative method. It is the opinion of the referee that a neutral baking powder would result and there would be no residual carbon dioxide if just enough bicarbonate of soda were added to react with the acid of the phosphate. The referee also believes that a correct method of determining the neutralizing value should show just how much bicarbonate of soda is required to obtain these conditions.

Since none of the methods thus far proposed fills this requirement, it is suggested that no action be taken on the tentative method at this time, but that an effort be made to obtain a method that will give the correct value.

The Committee on Recommendations of Referees last year suggested that the present referee study the two official gravimetric methods for the determination of carbon dioxide in baking powders<sup>1</sup>, with a view to determining whether both are used by analysts.

The information obtained by the referee is that the Knorr method is used quite generally and is accepted as being accurate, while the Heidenhain method is used only by a small number of persons and only as a standard reference method. The method is complicated but is capable of yielding very accurate results, owing to certain of its refinements not found in other methods; for this reason it is believed that it should be retained in the revised *Book of Methods* with a note stating that it is an extremely accurate method, intended primarily as a standard reference method, but inappropriate for routine work.

The referee gratefully acknowledges the assistance given by the Rumford Works and the R. B. Davis Company in furnishing the baking powder for collaborative study and wishes to thank the various collaborators for their splendid cooperation and their various suggestions and criticisms.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 301.

RECOMMENDATIONS<sup>1</sup>.

The referee has suggested certain changes in the chapter on Baking Powder and Baking Chemicals to the Revision Committee of the *Book of Methods* and desires authorization from this association to make certain other changes to improve the chapter. Therefore it is recommended—

- (1) That Section 2 be deleted.
- (2) That in Section 11, the alkali be changed to sodium hydroxide from potassium hydroxide.
- (3) That Sections 15 and 16 be deleted as they have been found to give erroneous results.

It is further recommended—

- (1) That additional collaborative data be secured on the electrolytic determination of lead.
- (2) That further collaborative determinations of carbon dioxide be made at approximately the same time and preferably as soon as samples are received.
- (3) That an attempt be made to secure a method of determining the neutralizing value of mono-calcium phosphate that will show the exact quantity of bicarbonate of soda required.

## REPORT ON FLUORIDES IN BAKING POWDER.

By JAMES K. MORTON (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

The collaborative work on the determination of fluorine in baking powder again emphasizes the difficulty, previously pointed out, of securing concordant results from different analysts.

The method prepared for the collaborators this year was identical with that of last year and seems to be as complete and explicit as it is possible to make it.

Three samples were submitted for analysis as follows:

Sample No. 1. Pure sodium fluoride.

Sample No. 2. A commercial grade of phosphate baking powder.

Sample No. 3. The same baking powder as No. 2 to which has been added 0.300 per cent of fluorine as sodium fluoride.

The results submitted on sodium fluoride by all the collaborators are good. The average recovery of fluorine by five analysts reporting on nineteen determinations was 91.0 per cent. This compares favorably with the results reported last year on sodium fluoride.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 272.



The results submitted on the baking powder samples show some wide variations. Four of the six collaborators are in fair agreement on all samples.

Milton H. Kemp suggests that the recovery of fluorine is lowered considerably if the sample contains more than 30 mg. of fluorine. It is true that more time is required to recover larger quantities of fluorine, but it is believed that the time limit of two hours in the method is more than sufficient to take care of any quantity of fluorine that a baking powder may contain. There is a probability, however, that more accurate and consistent results may be obtained on a smaller sample. This remains to be determined.

#### RECOMMENDATIONS<sup>1</sup>.

In view of the results thus far submitted on the determination of fluorine in baking powder, the associate referee recommends the continuation of this method as a tentative method.

It is also recommended that further collaborative and experimental work be conducted next year.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 273.

*Determination of fluorine in baking powder.*

COLLABORATOR	SAMPLE NO. 1—SODIUM FLUORIDE Fluorine calculated, 43.24 per cent			SAMPLE NO. 2—BAKING POWDER Fluorine calculated, 0.0575 per cent*			SAMPLE NO. 3—BAKING POWDER Fluorine calculated, 0.3575 per cent†		
	No. of Deter- minations	Found	Average recovered	No. of Deter- minations	Found	Average recovered	No. of Deter- minations	Found	Average recovered
		per cent	per cent		per cent	per cent		per cent	per cent
Milton H. Kemp Calumet Baking Powder Co., Chicago, Ill.	1	41.28	91.71	5	0.0585 0.053 0.0505 0.058 0.0577		3	0.376 0.362 0.342	100.6‡
						95.6	1	0.334	93.4§
J. L. Howerton Federal Phos. Co., Anniston, Ala.	3	43.6	96.5	2	0.0794	138.0	3	0.210 0.215 0.255	63.4**
T. J. Scott Federal Phos. Co., Anniston, Ala.				2	0.0795	138.0	2	0.3061	85.6
L. D. Mathias Victor Chemical Co., Chicago, Ill.	3	38.9	86.0	3	0.038	66.0	3	0.141	39.4
F. L. Thayer Rumford Chemical Co., Providence, R. I.	6	37.24 38.95 38.95 39.90 42.75 41.23	88.0	6	0.31 0.032 0.24 0.10 0.064 0.064	234.7	3	0.13 0.32 0.33	74.4
James K. Morton	5	39.8 42.8 44.13 39.92 43.4	92.9	3	0.049 0.054 0.049	88.1	4	0.2998 0.2864 0.3175 0.3090	84.7

\* Fluorine content calculated on average results of Collaborators 1 and 6.

‡ Determinations made on 10 gram sample.

† Fluorine added as sodium fluoride, 0.300 per cent.

§ Determinations made on 20 gram sample.

‡ Determinations made on 5 gram sample.

## DRUG SECTION.

### REPORT ON DRUGS.

By ARTHUR E. PAUL (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Referee*.

The work of this association on drugs, during the present year, has been planned along the general lines that were followed for the past several years. The plan contemplates the appointment of an associate referee for each important topic that should be given attention. Of these, a certain number were given consideration by the former referee, who devoted attention primarily to the topics that were most important, but for which no satisfactory methods had been adopted by the association. Most of these were retained during the present year, although a few changes were deemed desirable.

The subject of Crude Drugs was discontinued because the referee, even after discussion with other drug men, was unable to devise or suggest a method of attack of this broad subject, and further because it was believed that the topic is rather too inclusive for a single associate referee to handle. It will be noted, however, that papain and chaulmoogra oil, which should be classed as Crude Drugs, were included for study this year.

Likewise, the subject of Alkaloids was considered too broad, and it was limited, therefore, to the alkaloids of ipecac. The subject of the Separation of Quinine and Strychnine was retained as heretofore. Radioactivity in drugs and waters was taken up as a new topic, and that of turpentine was discontinued, because it is the understanding that this subject will be taken up under the general heading of Naval Stores.

All the work during the past year has been done largely by collaborators under the direction of the associate referees, and the results and suggestions for future work are included in the reports submitted by these associates.

Relative to recommendations on the subject of Phenolphthalein, a slight change seems desirable. To adopt the present tentative methods as official seems to be justified, but it is believed that the associate referee's method for chocolate mixtures can be adopted at this time only as a tentative method, with a view to its final adoption at a later time.

### RECOMMENDATIONS FOR FUTURE WORK.

It is desired to make the following general recommendations for consideration by next year's referee:

(1) That the following topics be studied by associate referees during the coming year:

Acetylsalicylic acid.  
Alcohol in drugs.  
Arsenicals (particularly sodium cacodylate).  
Camphor and monobromated camphor.  
Chaulmoogra oil.  
Chloramine T products.  
Chloroform and chloral hydrate.  
Ipecac alkaloids.  
Laxatives and bitter tonics.  
Mercurials.  
Papain.  
Pyramidon.  
Quinine and strychnine, Separation.  
Radioactivity in drugs and waters.  
Silver proteinates.

(2) That the following topics be discontinued for the present:

Barbital and phenobarbital.  
Methylene blue.  
Phenolphthalein.  
Phenylcinchoninic acid (atophan).

(3) That the following new topics be given consideration by the referee and that associate referees be appointed:

Nitroglycerin (Devarda method).  
Apomorphine.  
Santonin.  
Ether.

## REPORT ON ACETYLSALICYLIC ACID.

By CHANNING W. HARRISON (U. S. Food and Drug Inspection Station, Baltimore, Md.), *Associate Referee*.

The 1924 report of Sub-committee B on Recommendations of Referees on acetylsalicylic acid<sup>1</sup> contained seven recommendations, six of which would have necessitated chemical study in order to dispose of them properly. Since this was obviously too ambitious a program to be undertaken in one year, it was necessary to select a portion of it for this year's collaborative work. Accordingly, Recommendations 1 and 4 were taken up, since these presented definite problems on which to concentrate.

Recommendation 1 directed that the method for separating and determining acetylsalicylic acid in mixtures containing also caffeine and acetphenetidin be submitted for collaborative study. Recommendation 4 directed that the referee try to devise a satisfactory method for the determination of free and combined acetic acid.

Since work of previous referees had indicated that there was little hope

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 271.

of obtaining a satisfactory method for determining free acetic acid if present in acetylsalicylic acid, the work was directed towards obtaining a satisfactory method for determining combined acetic acid.

The two methods available to the referee for this determination, one by Paul and Elliott<sup>1</sup> and the other by Harrison<sup>1</sup>, were selected for collaborative study.

Four samples were prepared for submission to the collaborators, as follows:

(a) *For study of two methods for the determination of combined acetic acid:*

No. I. Acetylsalicylic acid (Mallinckrodt).

No. II. Commercial 5 grain aspirin tablets, powdered.

(b) *For study of determination of acetylsalicylic acid in mixtures containing caffeine, etc.:*

No. III. Powdered commercial tablets stated to contain per tablet—

	<i>gram</i>
Acetphenetidin.....	0.1176
Acetylsalicylic acid.....	0.1764
Caffeine.....	0.0294

No. IV. Powdered commercial tablets stated to contain—

	<i>gram</i>
Acetylsalicylic acid.....	0.225
Phenacetin.....	0.16
Caffeine.....	0.032

The average net weight of these tablets was found to be respectively 0.4092 and 0.4856 gram, which, on the basis of the stated composition, would correspond to 43.1 and 46.5 per cent of acetylsalicylic acid. The actual composition of these tablets, however, was not known.

Along with these samples the following instructions and methods were sent to the collaborators.

#### INSTRUCTIONS TO COLLABORATORS.

Samples designated No. I and No. II are to be used for the determination of total acetic acid by the two methods, and Samples No. III and No. IV are for the determination of acetylsalicylic acid in mixtures where caffeine and phenacetin are present, according to the enclosed method.

Sample No. I contains no excipient and therefore requires no preliminary extraction. In this case merely weigh out the required quantity of sample and proceed with the saponification.

Sample No. II is to be extracted and the percentage of chloroform extract determined preliminary to determination of the acetic acid by the two methods. Report the quantity of chloroform extract and acetic acid found.

In the case of No. III and No. IV, report percentage of total salicylic acid found and its equivalent percentage of aspirin. Make no correction for free salicylic acid.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 11.

It is requested that the work be carried out as soon as possible as it is feared that the samples may undergo deterioration if kept too long.

Your full criticisms and suggestions are requested on all methods, particularly your opinion of the relative merits of the two methods for determining total acetic acid.

## METHODS.

### *Quantitative Methods for Combined Acetic Acid.*

#### *No. I—Paul-Elliott Method.*

If excipients are present, treat 1 gram with a small portion of chloroform, filter into a weighed beaker, and wash until completely extracted. Evaporate the chloroform on the steam bath, dry in oven for 15 minutes at 80°C., weigh, and report the percentage of chloroform extract. To the extract in the beaker, add 15 cc. of normal sodium hydroxide and evaporate nearly to dryness. If no excipients are present, evaporate 1 gram and the 15 cc. of the normal hydroxide directly on a steam bath nearly to dryness. Transfer to a separatory funnel, using for this operation, successively, 10 cc. of water, 10 cc. of dilute sulfuric acid (10 per cent by volume), a small quantity of chloroform, and finally an additional 5 cc. of water. Shake successively with 30, 25, 20, 15, and 15 cc. of chloroform. Wash the combined chloroform extractions with three portions of water, 3 cc. each. Shake the original aqueous solution with an additional 10 cc. of chloroform and add this chloroform to the 9 cc. of wash water. Add the chloroform to the main bulk of chloroform. (This chloroform extract contains all the salicylic acid in the sample and may be used for its determination, but ordinarily it is preferable to determine the salicylic acid in a separate portion. Under these conditions the chloroform extracts may be discarded.)

Transfer the main bulk of aqueous solution to a 500 cc. flask, rinsing first with the 9 cc. of wash water and finally both separatory funnels with small portions of water. Steam distil into 60 cc. of 0.1 *N* alkali until acetic acid has been distilled over. This usually requires 500–700 cc. During the distillation keep the bulk in the distilling flask down to 20–25 cc. If the modified Hortvet volatile acid apparatus<sup>1</sup> is available, it is suggested that results obtained by its use be compared with those obtained by the use of an ordinary 500 cc. flask. Titrate the distillate with 0.1 *N* acid, using phenolphthalein as indicator.

1 cc. of 0.1 *N* alkali = 0.006003 gram of acetic acid.

#### *No. II—Harrison Method.*

Use 2 grams of sample. Weigh the sample and transfer to a separatory funnel with about 25 cc. of water. Extract the aspirin with six portions of chloroform, using 30, 25, 20, 10, 10, and 5 cc. portions. Pass each chloroform fraction through a plug of cotton inserted in the stem of a funnel, collecting the chloroform solution in a beaker of 150 cc. capacity that has been previously weighed and using a counterpoise beaker of the same capacity. Evaporate the chloroform to dryness on the steam bath and dry the extract for 15 minutes at 80°C. Cool in a desiccator and weigh. (The counterpoise beaker should be similarly heated and cooled before weighing.) Report the percentage of chloroform extract.

To the extract in the beaker add 30 cc. of normal sodium hydroxide and evaporate on the steam bath to approximate dryness. Wash this residue into a separatory funnel with about 20 cc. of hot water and then with about 10 cc. of dilute sulfuric acid (62 cc. of concentrated H<sub>2</sub>SO<sub>4</sub> made to 1 liter), continue the addition of dilute acid until the product is just acid, and then add 5 cc. excess.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1921, 5: 167.

Rinse the beaker with 50 cc. of chloroform into the separatory funnel and shake out the solution, drawing off the chloroform into another separatory funnel. Make four more extractions, using 20, 15, 10, and 5 cc. portions of chloroform, collecting these in the separatory funnel. Wash these chloroform residues twice with 20 and 10 cc. portions of water, collecting the water in a third separatory funnel, where it is washed once with 5 cc. of chloroform. Add the wash water to the acid water in the first separatory funnel.

During the extractions keep the stopper in the funnel to guard against loss of acetic acid by evaporation.

Transfer the water solution, which now contains the acetic acid, from the aspirin and excess of sulfuric acid to a glass stoppered 200 cc. volumetric flask and rinse the funnel several times with water to remove all the acid into the flask; make to volume; and mix thoroughly.

Pipet off two 50 cc. portions, using the same pipet drained the same length of time for each portion. Receive one portion in a suitable receptacle for titration and the other in a large platinum dish.

Titrate the first portion at once with 0.5 *N* alkali and phenolphthalein. Evaporate the second portion in the platinum dish on the steam bath, take up in 10 cc. of water, and again evaporate to dryness, repeating this process a second time. (During this evaporation guard against contact with ammonia vapors.) Take up in water, transfer to a suitable receptacle, and titrate the fixed acids with 0.5 *N* alkali and phenolphthalein. Subtract the second result from the first and calculate the percentage of acetic acid on 0.5 gram of sample.

1 cc. of 0.5 *N* alkali = 0.030015 gram of acetic acid.

*Method for Acetylsalicylic Acid in Mixtures Containing Acetphenelidin and Caffeine.*

Ascertain the average weight of tablets. Dissolve 0.200 gram of the powdered tablets in a small beaker with 10 cc. of chloroform and filter into a 200 cc. Erlenmeyer flask. Wash the beaker with successive small portions of chloroform until the chloroform-soluble material is completely exhausted. Evaporate on a steam bath until the volume is reduced to about 2 cc. Add 10 cc. of sulfuric acid (1 + 9). Connect with a reflux condenser and digest for one-half hour with the flask partially immersed in a boiling water bath. Wash the reflux condenser with small quantities of chloroform and water. Cool, and transfer to a separatory funnel with a minimum quantity of water so that the final volume does not greatly exceed 20 cc. Extract the caffeine and the salicylic acid with five portions of chloroform, using 30, 20, 10, 10, and 10 cc. To the combined chloroform extractions, add 20 cc. of water, then 1 gram of anhydrous sodium carbonate. Shake thoroughly. Transfer the chloroform to a separatory funnel, wash with 10–15 cc. of water, reject the chloroform, and combine the sodium carbonate solution and wash water in a 200 cc. Erlenmeyer flask. Heat on the water bath to expel traces of chloroform, dilute to 100 cc., add slowly 25–40 cc. of strong (about 0.2 *N*) iodine solution—sufficient to insure an excess during digestion—and digest for an hour on a steam bath. Remove the free iodine with a few drops of sodium thiosulfate solution, decant the clear liquid through a tared Gooch, retaining most of the precipitate, tetriodophenylenequinone ( $C_6H_2I_2O$ )<sub>2</sub>, in the flask. To the latter, add 50 cc. of boiling water, digest 10 minutes on the steam bath, filter, and wash gradually all the precipitate into a Gooch, using for this purpose and the final washing about 200 cc. of hot water. Dry to constant weight in an air bath at 100°C. Multiply the weight of the precipitate by 0.4016 to obtain the total salicylic acid and deduct the free salicylic acid previously determined. The difference represents the combined salicylic acid. Multiply by 1.304 to obtain the weight of aspirin.

The samples and methods were forwarded to seven collaborators, and results were received from three of these, as follows:

E. K. Nelson, Bureau of Chemistry, Washington, D. C.

W. F. Kunke, Bureau of Chemistry, Washington, D. C.

C. K. Glycart, Food and Drug Inspection Station, Chicago, Ill.

The results obtained by the collaborators on Samples I and II by the two methods for determination of combined acetic acid are presented in Table 1.

TABLE 1.  
*Collaborative results on combined acetic acid.*

SAMPLE	NELSON	KUNKE	GLYCART	HARRISON
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SAMPLE NO. I				
Paul-Elliott Method . . . . .		28.93 23.96* 32.86 33.08 29.05 30.44		28.51 31.22
	29.71		31.10	
Average . . . . .	29.71	30.87	31.10	29.87
Harrison Method . . . . .		33.24 33.60	31.58 33.85	30.62 31.27 32.56
	32.84			
Average . . . . .	32.84	33.42	32.72	31.48
SAMPLE NO. II				
Paul-Elliott Method . . . . .		24.50 24.32 24.70 23.95		19.21† 19.81
	23.81			
Average . . . . .	23.81	24.37		19.51
Harrison Method . . . . .		25.71 25.93 25.85	23.65 24.85	23.52 24.01
	25.64			
Average . . . . .	25.64	25.83	24.25	23.77

\* Omitted from averages.

† Chloroform extraction apparently incomplete.

#### COMMENTS BY COLLABORATORS.

*Nelson.*—The Harrison method is preferred as it is quicker and probably quite as accurate.

*Kunke.*—(Sample I—Paul-Elliott Method).—Determination No. 1, total distillate 650 cc., the last 150 cc. yielded 0.66 per cent acetic acid. Distillate No. 3 found to contain sulfuric acid. No. 4 also contained a trace. Distillate No. 5, measured 700 cc., required six hours of distilling. Blanks conducted with the apparatus and 700 cc.



distillate showed acidity equivalent to 0.0274 gram of acetic acid. The Paul-Elliott method is faulty mainly because no provision is made to exclude carbon dioxide from the distillate. I prefer the Harrison method to the Paul-Elliott method.

*Glycart.*—Previous experience with Method No. I for total acetic acid showed that low and variable results were obtained. Only one determination was made this year. Method No. II appears to give better results.

*Harrison.*—Lower results were obtained by the Paul-Elliott method than by the Harrison method. This is due, partially at least, to the method of extracting the acetylsalicylic acid from the dry powder, the dry method of extraction giving lower results than when extracting from a water solution.

The results obtained by collaborators on Samples III and IV were as follows:

TABLE 2.

*Collaborative results on the determination of acetylsalicylic acid in mixture containing acetphenetidin and caffeine.*

SAMPLE	NELSON	KUNKE	GLYCART
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
No. III.....		43.15 43.53	
	45.19	43.07 43.46	43.82 44.10
Average.....	45.19	43.30	43.96
No. IV.....		39.77 40.58	
	41.92	40.53 40.11	42.57 42.81
Average.....	41.92	40.25	42.69

*Glycart.*—(Samples III and IV) — \* \* \* It is my opinion that low results may be obtained due to the operation of dissolving the aspirin compound in chloroform and filtering. May I suggest that the following modification be applied: Transfer 0.200 gram of the powdered tablets to a small separatory funnel; dissolve in the minimum quantity of water (5 cc.); and extract with chloroform, using 25, 20, 15, 10, and 5 cc., or sufficient to remove completely. Combine the extractions in a 200 cc. Erlenmeyer flask.

#### DISCUSSION OF RESULTS.

Comparison of collaborative results by the two methods for combined acetic acid shows that Method II gives higher and more uniform results than Method I, and that on pure acetylsalicylic acid it yields results nearer the theoretical. All the collaborators express a preference for Method II, based on the fact that it is simpler and shorter, gives more uniform results, and appears to be fully as accurate, if not more reliable, than Method I. It is the opinion of the referee, however, that both methods possess sufficient merit to warrant their retention; therefore they will be presented in a rewritten form with a recommendation for their adoption provisionally.

Method I can be improved by changing the directions for extracting the acetylsalicylic acid with chloroform and also distilling over the acid into a clear flask protected to prevent evaporation and then titrating the distillate with standard alkali. Further, it would be advisable to recommend a uniform type of distillation apparatus, which would insure that sulfuric acid was not being carried over mechanically.

Following is the rewritten form of the method, such changes having been made as were suggested from the results of this year's work.

#### METHOD I.—REVISED.

*Quantitative Method for Combined Acetic Acid in Acetylsalicylic Acid by A. E. Paul and F. T. Elliott.*

In the case of tablets, determine accurately the average weight of a number of tablets; grind to a fine powder; and, if excipients are present, treat as follows:

Weigh accurately 1 gram of the powdered material and transfer to a separatory funnel, using about 25 cc. of water. Then extract completely with chloroform, testing the last extraction by evaporating a small quantity of the chloroform to dryness to be sure that the extraction is complete. Usually six extractions will be sufficient if 30, 25, 20, 10, 10, and 5 cc. portions of chloroform are used. Collect the chloroform fractions in a beaker and filter through a plug of cotton into a weighed beaker, which should have been weighed previously with a counterpoise beaker of the same dimensions, similarly dried and exposed to the air.

Wash the original beaker, funnel, and cotton with chloroform and add these washings to the chloroform solution contained in the weighed beaker.

Evaporate the chloroform on the steam bath, dry the chloroform extract at 80°C. for 15 minutes, and weigh, using the counterpoise beaker similarly treated. From the weight calculate the chloroform extract.

Treat the chloroform extract, or, if no excipients are present, 1 gram of the powdered material, in a 150 cc. beaker with 15 cc. of normal sodium hydroxide and evaporate on the steam bath nearly to dryness. Transfer to a separatory funnel, using for this operation, successively, 10 cc. of water, 10 cc. of dilute sulfuric acid (62 cc. of concentrated acid to 1 liter of water), and finally two 5 cc. portions of water. Extract with successive portions of chloroform, using the first fraction to wash out the beaker in which the saponification was carried on. Continue the extractions with chloroform until all salicylic acid is removed. (This generally requires about six extractions.) During these extractions keep the stopper in the funnel to guard against loss of acetic acid by evaporation. Collect the chloroform fractions in a second separatory funnel and wash with 25 cc. of water and this wash water once with 5 cc. of chloroform. Discard chloroform extractions and return the wash water to the acid water in the first funnel. Transfer the wash water to a 500 cc. distilling flask, washing both funnels carefully with several small portions of water to remove completely the acid and transfer again to the distillation flask. Subject to steam distillation until the volatile acids are completely distilled over. (This requires about 600 cc. of distillate.) Receive the distillate in a flask, placing a plug of cotton in the neck above the orifice of the condenser to prevent loss of acetic acid through evaporation. Use a distilling apparatus of such a type as to prevent the carrying over mechanically of sulfuric acid but keep the volume of liquid in the distilling flask at about 25 cc. (The modified Hortvet distillation apparatus has been found to be very satisfactory.) When 500 cc. of distillate has passed over, titrate with 0.5 *N* sodium hydroxide, using phenolphthalein as indicator. Continue the distillation until 100 cc. of distillate requires not more than 1 drop of 0.5 *N* sodium hydroxide to produce a distinct pink color in the presence of phenolphthalein. The total titration figure  $\times 0.030015$  = grams of acetic acid.

## METHOD II.—REVISED.

*Quantitative Method for Combined Acetic Acid in Acetylsalicylic Acid.*

(By C. W. Harrison.)

Weigh 2 grams of sample and proceed with the extraction with chloroform, saponification with 1 *N* sodium hydroxide, acidification with sulfuric acid, and extracting of salicylic acid with chloroform and washing of chloroform extract exactly as described in Method I, except to use 30 cc. of alkali for the saponification and to add about 5 cc. excess of dilute sulfuric acid over the quantity necessary to render the solution acid. (In extracting the salicylic acid from the acidified solution it is advisable to use 50 cc. of chloroform for the first extraction.) Transfer the acid water containing acetic and sulfuric acids to a 200 cc. graduate flask, wash the separatory funnels thoroughly with water, add to the flask, which is then made to volume with water, and mix thoroughly.

Pipet off two 50 cc. portions, using the same pipet drained the same length of time. Receive one portion in a receptacle suitable for titration and the other in a large platinum dish.

Titrate the first portion at once with 0.5 *N* alkali and phenolphthalein. Evaporate the portion in the platinum dish on the steam bath to dryness, take up in 10 cc. of water, and again evaporate, repeating this process twice more. During evaporation guard against contact with ammonia vapors. Take up the residue in water and titrate with 0.5 *N* alkali and phenolphthalein. Subtract the second titration reading from the first and calculate the percentage of acetic acid on a 0.5 gram sample.

1 cc. of 0.5 *N* alkali = 0.030015 gram of acetic acid.

The results given in Table 2 on the determination of acetylsalicylic acid in mixtures show that the collaborators obtained fairly concordant results.

Since the collaborators offered no criticisms of the method, it can be assumed that they found the technique in the main satisfactory. It is believed that Glycart's suggestion that the extraction be made with chloroform in a separatory funnel from a water menstruum is a good one and is in agreement with the experience of the referee that a liquid extraction with chloroform is more satisfactory than a dry one. It will be recommended, therefore, that this change be made in the details of this method. The amended method should then read as follows:

*Method for Acetylsalicylic Acid in Mixtures Containing Acetphenetidin and Caffeine.*

Ascertain the average weight of a number of tablets and reduce to a fine powder.

Weigh out accurately 0.2 gram of powder, transfer to a separatory funnel with about 25 cc. of water, and extract carefully with repeated portions of chloroform. Test the final extraction by evaporating a small portion on the steam bath to dryness. (No residue should remain if the extraction is complete. About six extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 cc. portions of chloroform.) Collect the chloroform fractions in a separatory funnel and draw off into a 200 cc. Erlenmeyer flask, placing a plug of cotton in the stem of the funnel to filter the chloroform. Wash the funnel twice with 5 cc. portions of chloroform, passing this through the cotton and leaving any water that may have separated in the funnel. Add the chloroform washings to the flask and evaporate the chloroform on the steam bath to a volume of about 2 cc. Add 10 cc. of sulfuric acid (1 + 9), connect with a reflex condenser, and digest for one-half hour, partially immersing the flask in a boiling water

bath. Cool, and transfer to a separatory funnel, rinsing the condenser with chloroform and using a minimum quantity of water to effect the transfer, so that the final volume does not greatly exceed 20 cc. Extract the caffeine and salicylic acid with six portions of chloroform, using 30, 25, 20, 15, 10, and 10 cc. for the extractions. Collect these fractions in a separatory funnel, add 20 cc. of water and 1 gram of sodium carbonate, and shake thoroughly. Drain off the chloroform into another separator and wash twice more with 15 and 10 cc. of water. Reject the chloroform and combine the sodium carbonate solution and wash waters in a 200 cc. Erlenmeyer flask. Heat on the steam bath to expel traces of chloroform, dilute to 100 cc. with water, then add slowly 25–40 cc. of strong iodine solution (about 0.2 *N*), sufficient to insure excess during digestion, and digest one hour on the steam bath. Remove the free iodine with a few drops of sodium thiosulfate solution. Decant the clear solution through a weighed Gooch having most of the precipitate in the flask. To the latter add 50 cc. of boiling water; digest 10 minutes on the steam bath; filter; and wash gradually all the precipitate into the Gooch, using altogether about 200 cc. of hot water to complete the operation. Dry to constant weight in an air bath at 100°C. and weigh the precipitate of tetraiodophenylenequinone ( $C_6H_2I_4O_2$ ). Weight of precipitate times 0.4016 gives the total salicylic acid present. If free salicylic acid is present, this should be deducted from the total, and the difference times 1.304 is the weight of acetylsalicylic acid.

#### RECOMMENDATIONS<sup>1</sup>.

As a result of this year's work the associate referee recommends:

(1) That the two methods for the determination of combined acetic acid, as rewritten in this report, be adopted as tentative methods.

(2) That the tentative method<sup>2</sup> for determining acetylsalicylic acid in mixtures, as slightly amended and rewritten in this report, be adopted as an official method.

(3) That since owing to the volatile nature of acetic acid little would be accomplished by the determination of free acetic acid in acetylsalicylic, the referee be excused from further work along this line.

(4) That the referee be excused from further work on acetylsalicylic acid in mixtures with interfering substances, such as was contemplated last year, since (1) in the majority of cases the present methods would probably be applicable, (2) in the case of more complex mixtures each mixture would constitute a research problem, and (3) the worth of such methods would not justify the time necessary to work them out—that is, they would be too restricted in nature to justify their existence.

(5) That the tentative method for the quantitative determination of free salicylic acid<sup>3</sup> be adopted as an official method.

(6) That the bromine method for the determination of total salicylates be given such study by the incoming referee as is necessary in his judgment to recommend its adoption as official.

(7) That the double titration method for the determination of acetylsalicylic acid<sup>4</sup> be studied by the incoming referee, as well as a simple

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 265.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 29.

<sup>3</sup> *Ibid.*, 1922, 5: 582.

<sup>4</sup> *Ibid.*, 583.

saponification method using alcoholic potash, and the results compared. The referee then may make the proper recommendation for the disposal of the double titration method.

(8) That the incoming referee study the relative merits of the "dry" and "wet" methods of extracting acetylsalicylic acid with chloroform and recommend a uniform procedure.

(9) That as the methods for acetylsalicylic acid, as now devised, are in the main satisfactory, collaborative study on this subject during the coming year be discontinued.

*C. O. Ewing:* While I have nothing to mention regarding this particular matter, I did have an experience with aspirin tablets several years ago that will be of interest to chemists generally. A particular lot of tablets during storage in an insufficiently dry atmosphere developed tiny spots and small cup-like depressions. Investigation proved that the condition was due to minute contamination with heavy metals, particularly iron, which apparently acted catalytically in the presence of moisture to hydrolyze the acid. The moisture concentrated at these foci and the resultant localized swelling of the starch caused the tiny depressions to develop. The condition could be duplicated experimentally.

As a result of this experience we have incorporated the following simple test into our specifications for aspirin:

Place about 10 grams of aspirin in a 250 cc. Erlenmeyer flask, moisten slightly with distilled water, and heat on a steam bath 3 hours. No distinct blue (Cu) or purple (Fe) spots should be visible.

No report on alcohol in drugs was given by the associate referee.

## REPORT ON METHODS FOR THE DETERMINATION OF ARSENIC IN SODIUM CACODYLATE.

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee.*

In connection with the A. O. A. C. report of 1922, Elias Elvove and C. G. Remsburg of the Bureau of Public Health called attention to the fact that the method for the determination of arsenic in arsphenamine<sup>1</sup> is not applicable to all organic arsenic compounds. A sample of sodium cacodylate that was known to contain about 33.5 per cent of arsenic showed only 4.3 per cent by this method. Since the assay in the U. S. Pharmacopoeia is based on the titration of the alkalinity of the solution

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 483.

with 0.1 *N* hydrochloric acid, it was recommended at the last meeting that a method be devised for the determination of arsenic in sodium cacodylate.

According to the description in the Pharmacopoeia, sodium cacodylate is sodium dimethylarsenate ( $\text{Na}(\text{CH}_3)_2\text{AsO}_2$ ), molecular weight 160.01, with a somewhat variable quantity of water of crystallization. It contains not less than 72 per cent nor more than 75 per cent of  $\text{Na}(\text{CH}_3)_2\text{AsO}_2$ . It occurs as white, odorless, deliquescent prisms or as a granular powder.

In search of a rapid and accurate method for arsenic the following attempts were made:

1. Digestion with potassium permanganate and strong sulfuric acid. The excess of permanganate was removed with hydrogen peroxide and, after neutralization with ammonia, the addition of ammonium nitrate, ammonium acetate, dilute acetic acid, and silver nitrate solution, no precipitate of silver arsenate resulted.

2. Fusion with sodium carbonate and potassium nitrate mixture and precipitation with silver nitrate, then weighed as  $\text{Ag}_3\text{AsO}_4$ , resulted in 70 per cent yield of the theoretical amount.

3. Fusion with sodium peroxide and precipitation with silver nitrate as silver arsenate. No results.

4. Digestion with strong sulfuric and nitric acids in a Kjeldahl flask, and precipitation by hydrogen sulfide in the presence of hydrochloric acid. The filtered arsenic sulfide was dissolved in strong sulfuric acid; after the sulfur dioxide had been expelled by prolonged heating, the neutralized solution was titrated in the presence of sodium bicarbonate with standard iodine solution<sup>1</sup>. Low results.

5. Digestion in a Kjeldahl flask with strong sulfuric and nitric acids to white fumes. The ammoniacal solution was treated with magnesia mixture. Precipitation incomplete.

6. Digestion in a Kjeldahl flask with strong sulfuric and nitric acids, precipitation with hydrogen sulfide in the presence of hydrochloric acid and weighing the arsenic pentasulfide in a Gooch crucible<sup>2</sup>. Approximate results.

#### DISCUSSION.

The results of analysis reported by Elvove were verified by the associate referee.

R. I. Grantham, Sharp and Dohme, Baltimore, Md., reported the following: "I have tried the oxidation with potassium permanganate and sulfuric acid without success. Sodium cacodylate is what we might consider a simple organic compound, and one might suppose, readily

<sup>1</sup> Sutton. Volumetric Analysis, 1911, p. 161.

<sup>2</sup> Treadwell. Analytical Chemistry, 1924, p. 205

oxidized by potassium permanganate, but this apparently is not the case. If the permanganate method could be so worked out in its detail that accurate results could be obtained it seems to me that this would be the most feasible. The Bennett modification of the Pearce method combining Volhard's would be suitable if it were not for the fact that loss is liable to occur in the process of fusion with potassium nitrate and sodium carbonate".

#### CONCLUSIONS.

(1) It is the opinion of the associate referee that sufficient work on the permanganate digestion has been done to show that it is not suitable for sodium cacodylate.

(2) The work on fusion methods was not sufficient to determine whether losses occur.

(3) Although hydrogen sulfide does not precipitate the arsenic in sodium cacodylate, a combination of first treating the compound with strong sulfuric and nitric acids then hydrogen sulfide may be useful in estimating the arsenic.

It is recommended that methods for the determination of arsenic in sodium cacodylate be further studied<sup>1</sup>.

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No report on phenylcinchoninic acid was made by the associate referee.

#### REPORT ON BARBITAL (VERONAL) AND PHENOBARBITAL (LUMINAL).

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation made at the last meeting, samples of barbital and phenobarbital were submitted to collaborators for further study this year.

The samples, which were purchased on the open market, consisted of veronal, luminal, and 5 grain veronal tablets. Directions for analyses and samples were sent to the following collaborators:

H. McCausland, The Abbott Laboratories, Chicago, Ill.

E. O. Eaton, Food and Drug Inspection Station, San Francisco, Calif.

S. Palkin, Bureau of Chemistry, Washington, D. C.

R. W. Hale, Food and Drug Inspection Station, Baltimore, Md.

The quantitative method has been published in *This Journal*<sup>2</sup>.

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<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 266.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 48.

*Results of analyses.*

COLLABORATOR	SAMPLE NO. 1—VERONAL				SAMPLE NO. 2—LUMINAL				SAMPLE NO. 3—VERONAL TABLETS	
	QUALITATIVE TESTS				QUALITATIVE TESTS				PERCENT-AGE	MELTING POINT
	QUANTI-TATIVE METHOD	1 Melting Point	2	3	4	1 Melting Point	2	3		
H. McCausland	per cent 100.03	°C. 190-191 corr.				°C. 175-176 corr.			81.74	
E. O. Eaton	100.1	190	Positive	Positive	Positive	175.4	Positive	Positive	81.7	
S. Palkin	99.8	195*				179*			81.97	195°C.*
R. W. Hale	100.25	190 188	Positive	Positive	Positive	172 171	Positive	Positive	82.87 82.70	191°C. Extracted material from tablets
C. K. Glycart	99.88	190	Indefinite	Indefinite	Indefinite	175	Indefinite	Indefinite	81.6	

\* Determinations made on micromelting point apparatus by J. F. Clevenger.



## QUALITATIVE TESTS.

*Barbital*.—(1) Melting point (Use U. S. P. method).

(2) To a solution of barbital acidified with nitric acid, add Millon's reagent.

(3) To a solution of barbital, add Deniges' reagent.

(4) Heat with sodium carbonate. Ammonia is liberated.

*Phenobarbital*.—(1) Melting point.

(2) Shake about 0.3 gram of luminal for a short time with 1 cc. of normal sodium hydroxide and 5 cc. of water. Filter the mixture. Divide the filtrate into two portions and add mercuric chloride solution to one and silver nitrate solution to the other. A white precipitate is given in each case. Boil about 1 gram of luminal for 5 minutes in 10 cc. of a 50 per cent solution of sodium hydroxide. Ammonia is evolved.

(3) Dissolve about 1 gram of luminal in 5 cc. of normal sodium hydroxide and heat the solution for 4 hours on a boiling water bath, replacing the evaporated water from time to time. Crystals of phenylethylacetyl urea separate on cooling. After recrystallization from dilute alcohol these crystals melt at 147°C.

## COMMENTS BY COLLABORATORS.

*R. W. Hale*.—The methods in the main are satisfactory. The slightly high results are probably due to remaining trace of sodium chloride as 2 cc. of water seems hardly enough to wash completely 90 cc. of solvent that contains 20 per cent of alcohol and consequently must contain an appreciable amount of salt solution. *Suggestion*: That a larger amount of water be used in washing and this in turn be washed with a small amount of chloroform to remove any veronal which it may dissolve.

*E. O. Ealon*.—It would appear that the qualitative tests were not characteristic, as under barbital, Tests No. 2 and No. 3 appear to be given by both substances.

*C. K. Glycart*.—It appears from results that the qualitative tests for barbital and phenobarbital, with the exception of the melting point, are not characteristic.

## SUMMARY.

The results obtained by the collaborators show that the quantitative method for barbital is quite satisfactory. Although the figures reported for phenobarbital are slightly higher than 100 per cent, the method is considered satisfactory.

As to the qualitative tests, the reported results on the determination of the melting point of barbital and phenobarbital are well within their respective range.

It is regretted that more collaborators did not report on the qualitative tests. It was especially desired to receive opinions concerning the results on adding Millon's and Deniges' reagents.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the method for the estimation of barbital and phenobarbital be adopted as a tentative method.

(2) That the procedure for melting point determination be adopted as a tentative method.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 266.

## REPORT ON CAMPHOR AND MONOBROMATED CAMPHOR.

By ARTHUR E. PAUL (U. S. Food and Drug Inspection Station,  
Chicago, Ill.), *Associate Referee*.

At the time suggestions were made by the Referee on Drugs for appointments of associates for camphor and monobromated camphor there was available only the unpublished report of a sub-committee of Committee B. This sub-committee suggested that the recommendations pertaining to both these subjects in the committee's report for 1922 be considered by the referee during the coming year and that suggestions be made for further study or deletion, as may be advisable. The available copy indicated that this had been approved, and the fact that subsequently the actual recommendation of Committee B included study of available methods was therefore overlooked. The instructions of the original sub-committee were accordingly followed by the associate referee, and careful consideration was given to the previous reports and recommendations on these two topics.

Relative to camphor, it does not seem that any methods have been studied by the association. In the 1921 meeting Gail H. Arner, as associate referee, gave a report on this topic<sup>1</sup> and mentioned, with references, the methods that had come to his attention. He included the following: a method involving determination by loss on volatilization; the hydroxyl amine method of Fuller and Nelson; various distillation methods with subsequent extraction with solvents, such as benzol or chloroform, and polariscopic readings of the solution; and the U. S. P. procedure, as applied to camphorated oil, which includes distillation in a current of alcohol vapor and polariscopic reading of the distillate. No actual work was done on any of these methods.

Relative to monobromated camphor, a report was made by Associate Referee C. D. Wright at the 1921 meeting<sup>2</sup>, in which he submitted and recommended for adoption two methods that were devised originally by W. O. Emery and E. O. Eaton. The methods are similar; they involve saponification, respectively, with sodium amalgam and alcoholic potash, with subsequent precipitation by silver nitrate and weighing of the precipitated silver bromide. The report of the associate referee, however, indicates the desirability of additional investigation. Both methods were tentatively adopted by the association, but with suggestions that they be given further study. This seems desirable, particularly since the collaborative work that has been done was performed on tablets purchased in the open market, concerning which, therefore, no positive information is available as to actual composition.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5, 544.

<sup>2</sup> *Ibid.*, 587.

During the two years following, 1922 and 1923, no work was performed, and the former recommendations were substantially repeated by the committee.

#### RECOMMENDATIONS<sup>1</sup>.

After reviewing carefully the available records relative to these two substances, it is respectfully recommended:

(1) That the Referee on Drugs for the coming year arrange for the appointment of an associate referee for the subject of camphor, and that the methods mentioned in Arner's report, together with any other available methods, be studied collaboratively.

(2) That the Referee on Drugs for the coming year arrange for the appointment of an associate referee for the subject of monobromated camphor, and that the present tentative methods of the A. O. A. C. and any other available methods be studied collaboratively with samples of known composition.

*Dr. Power:* While the paper on monobromated camphor was being read, it recalled many interesting recollections that may be of interest. I believe I had the pleasure of producing the first specimen of pure monobromated camphor made in this country, and that particular specimen, which was made 52 years ago, is now in the National Museum. It was deposited there with the idea that it would be of interest to future generations. I became interested in it through its use as a remedy by Dr. Hammond of New York, who at that time was one of the most noted nerve specialists in this country. I was then in charge of the pharmacy of Professor Edward Parrish of Philadelphia, and Professor John M. Maisch of the Philadelphia College of Pharmacy asked me to undertake the manufacture of this chemical.

*Chairman:* The next topic, chaulmoogra oil, is a new one. As much work had been done on this subject by a member of the Bureau of Chemistry, Dr. Frederick B. Power, he was requested to assist us. On account of his numerous other duties he recommended L. E. Warren of the American Medical Association as associate referee. Dr. Warren was appointed, and I think as he presents his report it will be found that Dr. Power's recommendation was a wise one.

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<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 266.

REPORT ON CHAULMOOGRA OIL<sup>1</sup>.

By L. E. WARREN (535 N. Dearborn Street, Chicago, Ill.),  
*Associate Referee.*

The seeds and oil of the kalaw (or chaulmoogra) tree have been used in India for medicinal purposes since very early times. The oil was used externally for skin diseases and internally in the treatment of leprosy. That the oil possessed specific curative properties in leprosy was known long before the mission of Gotama, but it never became very successful in treatment because of the irritation it produced in the intestinal tract of most patients. For this reason, its administration could not often be carried out for a sufficient time to produce cures.

About twenty-five years ago, clinicians began to search for methods of administering the oil by which the intestinal irritation could be mitigated, and a little later chemists and pharmacologists sought for derivatives of the oil that would be less toxic than the parent substance when given continuously over long periods. Progress was slow at first, but gradually several systems, which are now being used with considerable success, were developed. The preparation most frequently employed is a mixture of the ethyl esters of the total fatty acids of the oil. These are generally given by deep intramuscular injection. An aqueous solution of the soaps prepared by saponification of the oil is also much used. The separated fatty acids are given internally to some extent as a supplement to other treatment.

Chaulmoogra oil has been deemed of sufficient importance in medicine to have gained admittance to the British Pharmacopoeia (1914) and to New and Nonofficial Remedies (1924). It is also to be described in the forthcoming revision of the United States Pharmacopoeia (U. S. P. X). A closely related oil (hydnocarpus oil) is described in the Japanese Pharmacopoeia (Edition 4).

The chemistry of chaulmoogra oil was worked out by Power and his associates a number of years ago<sup>2</sup>. The oil differs markedly from the ordinary fixed oils. In addition to small quantities of the glycerides of the fatty acids commonly found in vegetable fats, chaulmoogra oil contains the glycerides of a series of highly unsaturated fatty acids, chiefly chaulmoogric acid,  $C_{18}H_{32}O_2$ , and hydnocarpic acid,  $C_{16}H_{28}O_2$ . This series of fatty acids differs from other ordinary fatty acids in being optically active and in possessing, as part of the molecular structure, a ring of carbon atoms.

<sup>1</sup> Since not all the analytical work outlined by the Associate Referee on Chaulmoogra Oil has been completed, this should be considered as a preliminary report. A more detailed and complete report will be submitted next year.

<sup>2</sup> Power and Gornall, *J. Chem. Soc.*, 1904, 85: 838, 851, Power and Barrowcliff, *ibid.*, 1905, 87: 884; and Barrowcliff and Power, *ibid.*, 1907, 91: 557.

True chaulmoogra oil is obtained from the seeds of *Taraktogenos kurzii* King (family Flacourtiaceæ). However, there are a number of plants that produce oils having a composition similar to chaulmoogra oil. A list of the principal species will be found in Table 1. The most important constants of these oils are included.

TABLE 1.  
*Constants of the oils of the chaulmoogra class compiled from various sources.*

SPECIES	SPECIFIC GRAVITY	POLARIZATION (+), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER
<i>Asteriastigma macrocarpa</i>	0.9519		98.38	202.7
<i>Hydnocarpus alcalae</i>	0.9500	49.6	93.1	188.9
<i>Hydnocarpus alpina</i>	0.9851	49.5	84.4 87.4	207.5 199.9
<i>Hynocarpus anthelmintica</i>	0.9530	49.5- 51.0	86.4 90.8	212 206.2
<i>Hydnocarpus castanea</i>			98.38	
<i>Hydnocarpus butchinsonii</i>	0.943		83.5	199
<i>Hydnocarpus subfalcata</i>	0.951	49.1 51.6	89 91.5	206 205
<i>Hydnocarpus venenata</i>	0.9471 0.9536 0.9550	52.3	99.7 97.6 99.1 99.69	202.7 200.3
<i>Hydnocarpus wightiana</i>	0.9548 0.9580	57.7	101.3	207
<i>Hydnocarpus woodii</i>		45.9	68.5	192
<i>Oncoba echinata</i>	0.8980 (at 100°)	48.8	96.5 99.7	192.4
<i>Taraktogenos kurzii</i> (True Chaulmoogra)	0.946-0.952	48-52	98-103.2	187-215

In 1921, F. L. Elliott was appointed Associate Referee on Chaulmoogra Oil. Owing to lack of time, no analytical work was done by him or his collaborators. His report<sup>1</sup> includes a comprehensive bibliography of the subject to 1922. At the close of 1923, the writer received notice of his appointment as Associate Referee on Chaulmoogra Oil. Four chemists accepted his invitation to do collaborative laboratory work on the oil. Including the associate referee, the names are given herewith:

<sup>1</sup> Unpublished.

C. L. Cox (C. L. C.), Valparaiso, Ind.  
 M. C. T. Katti (M. C. K.), Urbana, Ill.  
 W. J. McGill and L. R. Wagener (McG. & W.), Ann Arbor, Mich.  
 H. W. Vahlteich (H. W. V.), Chicago, Ill.  
 L. E. Warren, Associate Referee (L. E. W.).

The results obtained by these workers, so far as reported on September 15, 1924, are included in this report. It was decided to purchase market specimens of chaulmoogra oil and to ascertain whether they would conform to the standards required by New and Nonofficial Remedies and by the United States Pharmacopoeia X, as tentatively prepared. For the sake of comparison, the requirements of the several books of standards are given in Table 2.

TABLE 2.  
*Various standards for chaulmoogra oil.*

STANDARD	SOURCE OF OIL	SPECIFIC GRAVITY 25°/25°C.	POLARIZATION, IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER
British Pharmacopoeia	Taraktogenos kurzii	About 0.940 at 45°	52	96-104	198-213	21-27
Japanese Pharmacopoeia	Hydnocarpus anthelmintica		48	80-90	195-215	7
New and Nonofficial Remedies	Taraktogenos kurzii	0.9500	48-60	98-104	198-213	10-25
U. S. Pharmacopoeia X (proposed)	Taraktogenos kurzii and Species of Hydnocarpus	0.9500	48-60	98-104	196-213	10-25

Three specimens of chaulmoogra oil, representing three market brands, were purchased. Each specimen was warmed to insure melting of the separated solid portions of the material, and the liquid was thoroughly mixed and divided into five approximately equal portions. One sample of each brand was sent to each collaborator. The following methods and directions were also sent to each collaborator:

#### TESTS RECOMMENDED FOR COLLABORATIVE STUDY.

##### SECTION I

Chaulmoogra oil has a tendency to solidify partially or completely at room temperature. If the specimen is solid or contains solid particles, it will be necessary before analysis to warm it until the solids have melted. The whole specimen should then be thoroughly mixed by shaking and allowed to cool to room temperature. It will probably be necessary to repeat this before each sample is taken. Consequently time may be saved by making a number of weighings from the same sample at one time. If the order in which the determinations are described below is followed, material will be conserved.

*Specific gravity.*—Determine the specific gravity of the oil at 25°/25°C., using a pycnometer. The temperature of 25°/25°C. is selected because chaulmoogra oil will probably be described in the U. S. Pharmacopoeia in time, and the temperature of 25°C. is that prescribed therein for taking the specific gravity of most substances.

*Optical activity.*—Weigh about 5 grams of the oil (if the oil is very dark in color, use only about 2.5 grams for the determination), dissolve in chloroform, and make to a volume of 50 cc. at 20°C. with more of the solvent. Observe the optical rotation at 20°C. in a 100 mm. tube. Calculate the specific rotatory power according to the formula—

$$\text{Polarization} = \frac{10,000 \times a}{L \times c}, \text{ in which}$$

$a$  = the angle of rotation observed with sodium light.

$L$  = the length of the tube in millimeters.

$c$  = the number of grams of active substance in 100 cc. of the solution tested.

Please report the actual weights taken, the reading in angular degrees observed, and the specific rotatory power as calculated.

#### *Acid Number.*

(a) Weigh about 5 grams of the oil, previously warmed and well shaken; dissolve it in 20 cc. of carbon tetrachloride; add 0.5 cc. of phenolphthalein solution, and titrate the free acid with half-normal alcoholic potassium hydroxide. Calculate the number of milligrams of potassium hydroxide consumed by each gram of material and report as the "acid number"

(b) Weigh about 1 gram of the oil, dissolve it in 20 cc. of a mixture of equal volumes of neutral alcohol and ether, add 5 drops of phenolphthalein solution, and titrate with 0.1 *N* potassium hydroxide to a pink color that persists for 15 seconds. Calculate the number of milligrams of potassium hydroxide consumed by 1 gram of oil and report the number as the "acid number".

(c) (Optional) Use the official method<sup>1</sup>, except that 10 grams is sufficient as a sample because of the unusually high acidity of chaulmoogra oil. This method is given herewith.

Weigh 10 grams of the oil into a flask; add 25 cc. of 95 per cent alcohol by volume, which has been neutralized with dilute sodium hydroxide solution, using phenolphthalein as an indicator; and heat to boiling. Shake the flask thoroughly in order to dissolve the free fatty acids as completely as possible. Titrate with 0.1 *N* potassium hydroxide, shaking thoroughly until the pink color persists after vigorous shaking. Calculate the number of milligrams of potassium hydroxide consumed by 1 gram of oil and report the number as "acid number".

*Iodine absorption number (Hanus number).*—Weigh about 0.250 gram of the oil and complete the determination according to the official method<sup>2</sup>. Report the number of centigrams of iodine absorbed by 1 gram of oil as the "iodine absorption number".

*Saponification number (Koettstorfer number).*—Weigh about 5 grams of the oil and complete the determination by the official method<sup>3</sup>. Report the number of milligrams of potassium hydroxide consumed by one gram of oil as the "saponification number".

The results from these tests, so far as they have been reported by the collaborators, are included in Table 3.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 250.

<sup>2</sup> *Ibid.*, 244.

<sup>3</sup> *Ibid.*, 246.

TABLE 3.

*Analyses of chaulmoogra oil by several collaborators.*

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER		
					(a)	(b)	(c)
C. L. C.	0.9523			204.6	31.72		
M. C. K.			104.8 104.9 105.3	200.9 201.1			
McG. & W.*	0.9523	43.70	198	198	30.82	30.85	
H. W. V.	0.9561	49.20	81.45	204.70	31.74	31.56	31.38
L. E. W.	0.9520 0.9520	51.41 51.50	102.60 102.55 102.48	203.72 202.52 203.34	30.77	30.45	
C. L. C.	0.9522			199.5	31.82		
M. C. K.			104.7 104.5 104.3	196.2 196.9 196.7			
McG. & W.†	0.9520	44.90	199	196	29.48	28.8	
H. W. V.	0.9544	50.10	81.44	204.05	30.08	29.82	29.64
L. E. W.	0.9515	50.52	101.16 101.06	198.68	29.09	29.13	
C. L. C.	0.9537			204.9	27.00		
M. C. K.			98.93 98.78 98.78	202.2 202.0 202.6			
H. W. V.‡	0.9532 0.9530	55.30 55.40	75.22 74.85	207.6 208.3	26.36 26.60	26.88 26.90	25.90 26.00
L. E. W.	0.9538	54.70 56.52	98.60 99.51 96.81	204.75 205.43 203.12	26.18 26.20	25.92 25.63	
L. E. W.§	0.9501	51.26	101.79	201.35	16.57	16.84	

\* Parke, Davis &amp; Co. brand.

† Eli Lilly &amp; Co. brand.

‡ Lehn and Fink brand.

§ An authentic specimen of chaulmoogra oil that had been expressed by Frederick B. Power from identified seeds of *Taraktogenos kurzii* King in 1917.

An examination of these findings reveals wide discrepancies in some of the results. For example, the very high iodine numbers obtained by McG. & W. compared with the low values obtained by H. W. V. can not be reconciled. Correspondence with the collaborators failed to provide a satisfactory explanation, but these values will undoubtedly be revised by further work. In the case of the McG. & W. findings the value reported is probably a typographical error.



From a study of the constants of chaulmoogra oil as recorded in the literature and, to a lesser extent as obtained by the analyses, it seemed probable that the tests and standards in New and Nonofficial Remedies, and as proposed for the U. S. Pharmacopoeia X, were inadequate to exclude admixtures with some foreign oils in appreciable quantities. The specific gravity of chaulmoogra oil is very high (0.950–0.960). No other oils used in medicine except castor oil (0.945–0.965) and croton oil (0.935–0.950) approach this density. Croton oil would be ruled out as an adulterant because of its toxicity. The most characteristic property of chaulmoogra oil is its high optical rotatory power ( $+43^{\circ}$ – $63^{\circ}$ ), but castor oil and croton oil are also optically active to some extent, the polarization values given lying between  $12^{\circ}$  and  $28^{\circ}$  for castor oil according to the literature. The iodine absorption numbers of the first two oils do not overlap (chaulmoogra 98–113, castor 83–88), yet it seemed possible to the associate referee that there could be prepared a mixture of chaulmoogra oil and castor oil that would fall within the limits of the standards of New and Nonofficial Remedies. Accordingly, a specimen of castor oil was purchased, and its constants were determined by the associate referee. A mixture of ninety parts of chaulmoogra oil, the constants of which were known, were then mixed with ten parts of the purchased specimen of castor oil. The chaulmoogra oil used was known to have a rather low iodine value, but at the time the mixture was prepared this was the only specimen available in sufficient quantity to prepare the required amount of the mixture. Even then, on account of insufficiency of material, this specimen was not sent to all the collaborators. For the sake of comparison, the findings from the specimen of chaulmoogra oil obtained from Power are included in Table 4.

TABLE 4.

*Comparison of the constants of castor oil, authentic chaulmoogra oil, and a market specimen of chaulmoogra oil.*

KIND OF OIL	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER (b)
Castor	0.95918	7.96	84.35	182.67	1.57
Authentic Chaulmoogra	0.95014	51.26	101.79	201.35	16.84
Market Chaulmoogra (Sample C)	0.9538	54.70 56.52	98.31	204.43	25.78

An aliquot portion of the chaulmoogra-castor mixture was sent to three collaborators besides the associate referee, with the request that the specimen be examined to determine whether it conformed to the standards proposed for the U. S. Pharmacopoeia X. The findings, so far as reported, are included in Table 5.

TABLE 5.

*Analysis of a mixture of chaulmoogra oil and castor oil.*

(Sample D. Chaulmoogra oil 90, castor oil 10)

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION, IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER		
					(a)	(b)	(c)
McG. & W.	0.9557	55.3	95.98	186.86	23.3	23.7	
H. W. V.		50.02	73.45	202	24.34	26.55	
L. E. W.	0.95418	51.50	95.34	201.78	23.92	23.33	
U. S. P. X (Proposed)	0.9500	43-60	98-104	198-213		10-25	

A new supply of chaulmoogra oil was received, and careful determination of its constants was made by the associate referee.

TABLE 6.

*Comparison of the constants of a market specimen of chaulmoogra oil with those of an authentic specimen of chaulmoogra oil, and with a specimen of castor oil.*

KIND OF OIL	SPECIFIC GRAVITY AT 25°/25°C	POLARIZATION (20°C), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER (b)
Chaulmoogra Oil (Sample G*)	0.9506	56.08	103.3	203.74	24.26
Authentic Chaulmoogra Oil	0.95014	51.26	101.79	201.35	16.84
Castor Oil	0.95918	7.96	84.35	182.67	1.57

\* Parke, Davis Brand No. 2.

As the specimen conformed to the standards proposed for the U. S. Pharmacopoeia X another specimen of sophisticated chaulmoogra oil was prepared, this brand being used as the source of the chaulmoogra oil. The second mixture contained 80.95 parts by weight of chaulmoogra oil and 19.05 parts of castor oil. A portion of the well mixed specimen was then sent to each of several collaborators, with the request that it be subjected to the tests in Section I, *i. e.*, those tests to be required by the U. S. Pharmacopoeia X. The findings are incomplete, but so far as reported they are given in Table 7.

While the results obtained from the sophisticated specimens are fragmentary, they indicate clearly the need for more elaborate tests and standards to prevent adulteration than are proposed by the U. S. Pharmacopoeia X. A comparison of the constants of most market oils reveals that they could not be used to adulterate chaulmoogra oil in any appreciable quantities because of their low specific gravity, generally low iodine absorptive power, and lack of optical activity. For example, if a specimen of chaulmoogra oil had a low iodine absorption value, this defect might

TABLE 7.

*Analysis of a specimen of chaulmoogra oil and castor oil.*

(Sample E. Chaulmoogra oil 80.95; castor oil 19.05.)

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER (b)
C. L. C.	0.9526			199	20.55
M. C. K.			100.3 100.8 100.6 101.0	195.4 195.2 196.0 195.7	
L. E. W.	0.9524	47.55	97.49 97.51	197.1 201.3	19.70 19.38
U. S. P. X (Proposed)	0.9500	43-60	98-104	198 213	10-25

be obviated by the judicious addition of linseed oil, but such a procedure would lead to a suspicious lowering of the optical rotation. It is probable that almost the only oils that could be used as adulterants in appreciable quantities without detection by the present standards are castor oil and

TABLE 8.

*Calculated values for standard chaulmoogra oil adulterated with 10 per cent each of castor oil, linseed oil, and tung oil.*

KIND OF OIL	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE ABSORPTION NUMBER	SAPONIFICATION NUMBER	ACID NUMBER
100 units Chaulmoogra oil	0.95014	51.26	101.79	201.35	16.84
100 units Linseed oil	0.930		170	190	
100 units Castor oil	0.95918	7.96	84.35	182.67	1.57
100 units Tung oil	0.9420 at 15°		152	194	6
90 units Chaulmoogra oil	0.85513	46.134	91.70	181.215	15.156
10 units Castor oil	0.09592	0.796	8.435	18.267	0.157
Total	0.95105	46.930	100.135	199.482	15.313
90 units Chaulmoogra oil	0.85513	46.134	91.70	181.215	15.156
10 units Linseed oil	0.09300	0.0	17.00	19.000	0.6
Total	0.94813	46.134	108.70	200.215	15.756
90 units Chaulmoogra oil	0.85513	46.134	91.70	181.215	15.156
10 units Tung oil	0.09420	0.0	15.20	19.400	0.600
Total	0.94933	46.134	106.90	200.615	15.756

croton oil. The second of these would not be used because of its high cost and great toxicity. Some of the drying oils might be added in amounts up to 8 or 10 per cent. Table 8 gives calculated values for chaulmoogra oil mixed with ten parts each of castor oil, tung oil, and linseed oil.

From a study of the constants of some of the possible adulterants, as recorded in the literature, it seemed likely that such adulterants might be ruled out by establishing standards for viscosity, percentage dissolved by a given volume of alcohol, and the loss or gain in weight of a film on standing or heating. Accordingly, the tentative tests given below were devised and sent to each of the collaborators. The determination of the index of refraction and tests for cottonseed oil and sesame oil were included.

## SECTION II.

*Viscosity (Optional).*—Determine the viscosity in a viscosimeter, using either a Saybolt Universal, Engler, or Ubbelohde instrument. If none of these instruments is available, use the U. S. Pharmacopoeia method for liquid petrolatum<sup>1</sup>. Report the kind of instrument used, as well as the values found.

*Loss or gain on heating.*—Weigh about 2 grams of the oil into a flat-bottomed dish, expose to the air in a warm place protected from dust for 24 hours, and weigh. Heat on a steam bath for 2 hours, cool, and weigh again. The oil should not materially gain in weight (*absence of foreign drying oils*) nor materially lose in weight (*absence of volatile oils*).

*Soluble in alcohol.*—Place 25 cc. of the oil in a Rose and Hertzfeld fusel oil determination apparatus, taking care that drops of oil do not adhere to the walls of the tube above the 25 cc. mark. Add 100 cc. of alcohol, stopper the tube, and shake thoroughly for 15 minutes. Allow the mixture to stand overnight and observe the reading of the lower layer. Calculate the percentage of oil dissolved by volume. (If the volume of undissolved oil does not reach the 20 cc. mark, slowly add more of the same sample of oil from a buret until the volume reaches 20 cc. Subtract the volume of oil added from 20 cc. The result is the volume remaining undissolved.)

Pour the mixture into a separator, draw off the oily layer, and evaporate the alcoholic solution on a steam bath to constant weight. Determine the iodine number of this alcohol-soluble substance and its acid number by the methods already used for the determination of these constants.

*Index of refraction.*—Determine the index of refraction by means of an Abbé refractometer according to the official method<sup>2</sup>.

*Cottonseed oil.*—Mix 5 cc. of the oil in a test tube with 5 cc. of a mixture of equal volumes of amyl alcohol and carbon disulfide, which contains 1 per cent of sulfur in solution, and immerse the test tube to one-third its depth in boiling, saturated aqueous salt solution; no reddish color develops in fifteen minutes (*absence of cottonseed oil*).

*Sesame oil.*—Mix 1 cc. of the oil with 1 cc. of hydrochloric acid containing 1 per cent of sugar, shake the mixture for half a minute, and allow to stand for 5 minutes; on adding 3 cc. of distilled water to the mixture and again shaking it, the acid layer shows no pink color (*absence of sesame oil*).

<sup>1</sup> U. S. Pharmacopoeia IX, p. 314.

<sup>2</sup> Assoc. Official Agr. Chemists, Methods, 1925, 282.

The collaborative work in Section II of the tests has not been completed by any of the collaborators. The reported results are given in Table 9.

TABLE 9.  
*Report on Section II of the collaborative tests.*

BRAND	COLLABORATOR	VISCOSITY U. S. P. IX	PERCENTAGE LOSS		PERCENTAGE SOLUBLE IN ALCOHOL	ND 20°	COTTON-SEED OIL TEST	SESAME OIL TEST
			24 hrs.	2 hrs.				
A	M. C. K.		0.043	0.052			Negative	Negative
	L. E. W.	14.06			9.6	1.4781		
B	M. C. K.		0.069	0.057			Negative	Negative
	L. E. W.	13.44			11.6	1.4772		
C	M. C. K.		0.056	0.010			Doubtful	Negative
	L. E. W.	15.66			9.2	1.4775		
D	L. E. W.	19.86			15.2	1.4770		
E	M. C. K.		0.081	0.042			Negative	Negative
	L. E. W.	20.31			24.0	1.4772		
F	L. E. W.	14.19			6	1.4772		
G*	L. E. W.	14.25			2.88	1.4782		
†	L. E. W.	78.25	Castor	Oil	100	1.4766		

\* Authentic specimen of chaulmoogra oil.

† Castor oil.

#### CONCLUSIONS.

The results are too incomplete to warrant any very positive conclusions being drawn from them. However, the *immediate* increase in viscosity and in the proportion of oil soluble in a given volume of alcohol in the mixtures containing castor oil are indicative that these tests will prove of value in the detection of this adulterant in chaulmoogra oil. The refractive index of chaulmoogra oil lies so near that of castor oil that this constant is not much changed by the admixture of the two oils. The tests by Katti indicate that volatile oils and drying oils were absent from the specimens on which he worked. So far as the tests indicate, all the specimens examined except those purposely sophisticated are of good quality. It is quite possible that some of them may be hydnocarpus oils or mixtures of hydnocarpus oil with chaulmoogra oil.

#### COLOR REACTIONS.

Very little collaborative work has been done on the color reactions of chaulmoogra oil. According to the literature the most satisfactory identity test is that described by Lifschutz<sup>1</sup>. This consists in dissolving

<sup>1</sup> Chem. Z., 1921, 45: 1264.

one drop of the oil in 0.5 cc. of chloroform, diluting with 1.5 cc. of glacial acetic acid, and adding four or five drops of sulfuric acid. There develops gradually a grass-green color that is reddish violet by transmitted light. It is proposed to study this and other color reactions during the coming year; therefore it is recommended that the collaborative work be continued<sup>1</sup>.

*Dr. Power:* Mr. Chairman: I am reminded that it was 20 years ago when I published my first paper on the composition of chaulmoogra oil and on the constitution of the principal acids contained therein, which are chaulmoogric and hydnocarpic acids. The characters of the acids have been referred to by Dr. Warren, and it may be recalled that the acid present in largest proportion, chaulmoogric, is isomeric with linolic acid, the well-known acid occurring in many oils, although linolic acid is liquid and chaulmoogric acid is solid. The difference between them is apparent in the fact that the chaulmoogric acid absorbs only two atomic proportions of bromine or iodine, showing that it contains only one ethylenic linking, whereas linolic acid, which absorbs twice that quantity, must have an entirely different constitution. Moreover, chaulmoogric acid is optically active, whereas linolic acid is inactive. These facts led to a study of the constitution of the acid, which required two or three years of work and proved it to be a hydrocarbon ring with a side chain of twelve methylene groups and one carboxyl group.

It is desirable that we should have every possible safeguard for the purity of such an important product as chaulmoogra oil, for I think we have the assurance that the ethyl esters of its fatty acids provide, in a large number of cases, a cure for leprosy. I think Dr. Warren has indicated that, in collaboration with Dr. G. D. Rosengarten, I was called upon to prepare a monograph on this oil for the U. S. Pharmacopoeia X, which has been accepted. An interesting question has arisen in connection with some other closely related oils, and especially those from species of *Hydnocarpus*, which belong to the same botanical family as the true chaulmoogra. *H. Wightiana* comes from Western India and *H. anthelmintica* chiefly from Siam and is exported to China. The new Japanese Pharmacopoeia has adopted one of these hydnocarpus oils. About 20 years ago I examined the two above-mentioned hydnocarpus oils and found that they agree very closely in composition with true chaulmoogra oil. It seems desirable, therefore, that we should include them in the Pharmacopoeia. It is perfectly right and proper that hydnocarpus oil should be used when it conforms to the required standard of purity, and in fact I believe it is used very largely in India in place of the true chaulmoogra oil for the preparation of the ethyl esters.

No report on chloramine-T products was made by the associate referee.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 267.

## REPORT ON CHLOROFORM IN DRUG PRODUCTS.

By H. O. MORAW (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee*.

The work this year on chloroform was a continuation of that of the last few years, and consisted in trying out the method based upon the decomposition of the chloroform by alcoholic alkaline solution and estimation of the chlorine by the Volhardt method.

The preliminary study by the associate referee consisted in trying out the method as modified by the suggestions and experience of some of the collaborators during 1923. One of these suggestions was to make comparison of the yield when using sodium hydroxide with the yield when using potassium. Another suggestion was to use larger quantities of alkali, such as 30 cc. instead of 10 cc. A comparison of the two alkalis on the same samples of chloroform showed that potassium gave 1.5-2 per cent higher yield than sodium hydroxide. This was later confirmed by the collaborators that tried it. The preliminary investigation of the method also indicated that by using methyl alcohol as a solvent for the sample instead of ethyl there resulted a higher yield. This was not confirmed by the collaborators nor by the later assay of the collaborative samples by the associate referee. It is assumed that the previous higher yield was due to errors in observing the end point of the titration.

The directions for preparing the reagent and for the procedure in the assay were revised in accordance with the above findings. It was considered that the development of the method had not reached the stage where it could be applied to the assay of chloroform in medicinal preparations such as liniments and cough sirups because its dependability in the hands of different chemists had not been established. The work this year, therefore, was confined to trying out the revised directions. Different collaborators used the same samples, which were prepared so as to be free from interfering substances. Samples of U. S. P. chloroform in the Chicago Station Laboratory labeled to contain not less than 99 per cent of chloroform yielded by this method 94-97 per cent. Repurification of these samples by two different methods did not result in higher yield of chloroform when assayed by this procedure. The chloroform used to make up the collaborative samples assayed 96-97 per cent by this method. Because of the volatility of chloroform and its liability to decompose, it seems advisable to describe the preparation of the collaborative samples.

One hundred grams of the U. S. P. chloroform (which assayed by this method 96-97 per cent) was quickly weighed in a 100 cc. flask, 5 milligrams in excess being allowed to compensate for evaporation during weighing and transferring. A little methyl alcohol was used to wash down the

neck of the flask; it was rapidly transferred to a liter graduated flask and dissolved in methyl alcohol; and 200 cc. of water was added and then made to 1 liter with methyl alcohol. This was Sample No. 1. For Samples 2 and 3, the same 100 cc. pipet was used for taking aliquots. No. 2 was made to a liter, methyl alcohol and 300 cc. of water being used, and No. 3 was made to a liter, ethyl alcohol and 300 cc. of water being used. These stock solutions were kept in brown glass bottles, and the collaborative samples were sent out in colorless glass 8 oz. bottles sealed with paraffined cloth.

*Collaborative results on chloroform by volumetric method.*

COLLABORATOR	SAMPLE No 1 10 GRAMS $\text{CHCl}_3$ PER 100 CC. IN $\text{CH}_3\text{OH}$ CONTAINING 30 PER CENT $\text{H}_2\text{O}$		SAMPLE No 2 1 GRAM $\text{CHCl}_3$ PER 100 CC. IN $\text{CH}_3\text{OH}$ CONTAINING 30 PER CENT $\text{H}_2\text{O}$		SAMPLE No 3 1 GRAM $\text{CHCl}_3$ PER 100 CC. IN $\text{C}_2\text{H}_5\text{OH}$ CONTAINING 30 PER CENT $\text{H}_2\text{O}$	
	REAGENT		REAGENT		REAGENT	
	KOH	NaOH	KOH	NaOH	KOH	NaOH
	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc
C. F. Whitney Vermont State Labor- atory of Hygiene, Burl- ington, Vt.	9.2336		0.8756		0.8358	
M. Garretson Lehn & Fink Research Laboratory, Bloom- field, N. J.	9.30 9.2019	8.20* 8.28	0.8706 0.8953	0.820* 0.820	0.9303 0.8953 0.9303	0.820* 0.819
M. Crane United Drug Co. Re- search Laboratory, Boston, Mass.	8.32		Lost		0.836	
A. W. Hanson U. S. Food and Drug Laboratory, Chicago, Ill.	9.3		0.81		0.90	
H. O. Moraw U. S. Food and Drug Laboratory, Chicago, Ill.	9.65† 9.59		1.003† 1.002		1.003† 1.003	
A. G. Murray U. S. Bureau of Chem- istry, Washington, D. C.	8.90	8.865‡	0.88	0.87‡	0.865	0.86‡

\* Collaborators were not asked to use NaOH as the reagent but to report results if convenient

† Samples stored in brown bottles. Other samples sent to collaborators in colorless bottles

‡ Saturated NaOH in methyl alcohol used as the reagent.



## METHOD.

## PREPARATION OF REAGENT.

*Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide in 30 cc. of water, cool, and add methyl alcohol to make 100 cc. If more than a trace of chloride is present, it should be determined and a correction applied to the chloroform determination.

## DETERMINATION.

Accurately measure 5–10 cc. of the sample, containing 0.005–1 gram of chloroform, into a 100 or 200 cc. graduated flask. Add 30 cc. of the alcoholic potassium hydroxide, stopper the flask, mix thoroughly, and allow to stand at room temperature overnight. Heat 10 minutes on a steam bath. Dilute to the mark with water and determine chloride in an aliquot by the Volhardt method. 1 cc. of 0.1 N  $\text{AgNO}_3$  = 3.98 mg. of  $\text{CHCl}_3$ . Report results in grams per 100 cc.

## COMMENTS BY COLLABORATORS.

*M. Garretson.*—Higher results were obtained with the potassium reagent than with the sodium reagent. By using several cc. of nitric acid when titrating, a clearer and sharper end point was obtained. Aside from this, the Volhardt method seems to be suitable.

*M. Crane.*—Apparently we get a much quicker action on the chloroform by dissolving the potassium hydroxide reagent with ethyl alcohol that has been purified by silver nitrate and potassium hydroxide, this solution affording effective results within a four hour period by heating at once.

*A. W. Hanson.*—A loss of chloroform may occur on heating the alkaline solution. This may be avoided by use of a reflux condenser. It might be possible to use sodium amalgam to decompose the chloroform. See methods for bromine in brominated camphor.

*A. G. Murray.*—I made the determinations also by saponifying with a saturated solution of sodium hydroxide in methyl alcohol. The results obtained with this latter reagent are slightly but uniformly lower than those obtained with potassium hydroxide. I am inclined to think, however, that the difference is due to the fact that the sodium hydroxide solution is not so concentrated as the reagent you suggest. In all probability sodium hydroxide dissolved in water and made up to volume with methyl alcohol in the same way you prepare the potassium hydroxide reagent would yield accurate results.

## DISCUSSION.

Considering the nature of chloroform, its volatility and liability to decompose, there is fairly good agreement in the results of all the collaborators except the associate referee, whose results were consistently higher than any of the others. Unfortunately none of the other collaborative samples were kept in colored bottles, so there were no other collaborative results on these samples to confirm the assumption that these high figures were due to the better keeping qualities of chloroform stored in colored bottles. The practice of manufacturers in shipping chloroform in colored bottles, together with this experience, should be taken as a guide in conducting further work on this substance.

The fact that the samples numbered 2 and 3, which were stored in brown bottles, yielded higher results might be due to the smaller quantities used for the determination. On this point more work should be done.

When the Volhardt method for chlorides is applied to this assay, a part of the nitric acid added for the precipitation of silver chloride is consumed in oxidizing some of the alcohol present. The result may thus be affected by lack of a proper amount of nitric acid during this reaction.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended that this method, with the suggestions and experience of the collaborators during this year, be studied for the coming year.

#### REPORT ON CHLORAL HYDRATE.

The work on the method for chloroform in drug products was undertaken first, and no time was available to devote to the subject of chloral hydrate.

#### REPORT ON IPECAC ALKALOIDS.

By A. R. BLISS, JR. (College of Medicine, University of Tennessee, Memphis, Tenn.), *Associate Referee*.

Though the associate referee has no report concerning the results of actual collaborative work accomplished during the past year, he desires briefly to outline the work started. It is hoped that the problems under way will be solved during the coming year.

The first investigation in the study of the gravimetric and the volumetric methods for the assay of ipecac and its preparations concerned itself with two methods of assay suggested by E. C. Merrill, C. K. Glycart, and the associate referee. Carefully prepared samples of the crude drug and of ipecac fluidextract were sent to six collaborators with the request that the following methods be carried out on both the drug and the fluidextract.

#### METHODS<sup>2</sup>.

(1) *Volumetric Method of the U. S. P. IX.*—Carefully follow the U. S. P. method for both crude drug and fluidextract. *Take extreme care to prevent loss of small quantities of alkaloids* during the extraction and throughout the process, and also to *avoid* possible decomposition of the alkaloids by *excessive heating*. In removing the last traces of solvent, the beaker should always stand in a level position while on the bath and should be removed from the heat while approximately 3 cc. of solvent remains, this last portion of solvent being evaporated by a jet of preheated air. Unless these precautions are observed, loss in alkaloids results owing to incomplete extraction or decomposition during evaporation. (See report for 1921<sup>3</sup>—portions relating to ipecac.)

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 266.

<sup>2</sup> U. S. Pharmacopoeia, IX, 1916, 187.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 570.

(2) *Gravimetric Method*.—Follow the assay procedure of the U. S. P. IX, but instead of dissolving the alkaloidal residue in acid, dry it to constant weight at a temperature not exceeding 95°C. (or, if possible, use a vacuum oven and a still lower temperature), such as might be obtained in a water jacketed oven. Please state the apparatus and the exact temperature employed in reporting your results.

Since two collaborators only reported the results of their investigation, it was decided to postpone a detailed report on the work undertaken until a majority of the collaborators had reported their findings.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that this study be continued.

*S. Palkin:* I have been awaiting the report on ipecac alkaloids with some interest, and I know that I am expressing the sentiment of the members of the Drug Control Laboratory in stating that we are all disappointed in the failure of the collaborators to report.

During the past half year this laboratory has been experimenting with various mechanical devices for the extraction of alkaloids from the galenicals. Among those that appear to lend themselves most readily to most of the types of extractors are the fluidextracts of ipecac, nux vomica, and belladonna. The results that we have had so far with the mechanical device tend to show that the total quantity of alkaloids extracted from the fluidextract is invariably higher than that obtained by the methods given in U. S. P. VIII, IX, or X. The device used for ipecac is of an exceedingly simple type and involves the use of ether as the extracting medium. A comparison of some of the results obtained might be of interest here, although I might say that the work is more or less preliminary, and at this stage it is impossible to draw conclusions as to the reasons for these differences. Now, while certain preparations yield 1.536 grams per 100 cc. by the U. S. P. IX method, the method given in U. S. P. X, which has not yet been issued, yields 2.04 grams, whereas by the mechanical extractor the three following results were obtained: 2.23, 2.23, and 2.22 grams. Therefore, any preparation that, in accordance with the U. S. P. IX method, might be 15 per cent short, and within the limits of U. S. P. IX when analyzed, according to the U. S. P. X method, was actually plus by the mechanical extraction method. Of the large number that we have taken that way, in many cases the total alkaloid obtained by any of the U. S. P. methods shows the content of the preparation to be deficient where the mechanical extractor device gave sufficient to make it actually high. The possibilities of decomposition of the various alkaloids present in ipecac of course enter into the consideration of any mechanical device that involves heating the extract to the point of solvent vaporization and subsequent condensation, but so far the evidence seems to point the other way, as all of the results seem high. When the subject has been studied thoroughly, we hope to be able to publish the results.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 267.

## REPORT ON RADIOACTIVITY OF DRUGS AND WATER.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The work described below was planned for this year:

1. To describe in detail the method used in the Bureau of Chemistry for determining radium by the emanation method and to include in the description a sketch of suitable apparatus, a bibliography, and the details of the method now employed for standardizing electrosopes. No cooperative work on the method, which is based on a recognized standard procedure, was contemplated.

2. To develop methods for preparing solutions or extracts of various types of drugs, such as pills, pad materials, suppositories, ointments, etc., alleged to be radioactive, with a view to determining their radioactivity.

A description of the procedure for determining the radium in clear solutions has been prepared and is given in this report, together with a sketch of an apparatus that is suitable for use, a selected bibliography, and a description of the method for standardizing electrosopes. While many data regarding the work contemplated in Paragraph 2 have been collected, the investigation has not been completed, and the report on it will necessarily be deferred until next year.

The procedure in detail for the determination of radium in clear solutions of drugs and water follows:

**METHOD.****REAGENTS.**

(a) *Sodium hydroxide solution.*—Dissolve 60 grams of sodium hydroxide in water, dilute to 3 liters, boil the solution at least 10 minutes, and keep hot until ready for use. Prepare the solution fresh each day.

(b) *Calcium chloride.*—Granular, anhydrous for drying.

(c) *Standard radium solution.*—(A description of a method of preparing the standard stock solution of radium is in course of preparation.) Prepare the standard, dilute solution as follows: Measure accurately such a quantity of the clear standard stock solution of radium as will contain about 5 millimicrograms of radium and place it in a flat-bottom Erlenmeyer flask of about 100 cc. capacity, provided with a sealed-in glass tube extending horizontally about 12 cm. If the volume of the standard solution taken is less than about 80 cc., make it up to about 80 cc. with a boiled solution of nitric acid (1 + 12) containing about 0.1 gram of barium nitrate per 100 cc. Record the exact quantity of standard solution taken and its temperature. Boil the diluted solution rather vigorously for 20 minutes, carefully avoiding mechanical loss; allow to cool somewhat; replace the solution lost by evaporation with an equal quantity of boiled water; and draw out and seal the end of the glass tube, recording the exact time of sealing off. Allow the solution to stand at least 4 days.

**APPARATUS.**

(a) *Reichardt's apparatus as modified by Boltwood and by the Bureau of Chemistry for the collection of gases dissolved in liquids.*—The apparatus consists of 2 flat-bottom,



long-neck Florence flasks, the one containing the sample having a capacity of from 0.1–2 liters and the other a capacity of 2 liters (Nos. 1 and 2 in Fig. 1); 2 overflow flasks of about 300 cc. capacity (3 and 4); 1 gas-collecting buret 4 cm. in diameter and 50 cm. in length (5); 2 gas burners; and 2 iron supports provided with iron clamps, 1 iron tripod, sufficient rubber and glass tubing, wire gauzes with asbestos centers, and pinch cocks. Arrange the apparatus as shown in Fig. 1.

(b) *Gas transfer apparatus*.—Consists of 1 gas transfer buret 4 cm. in diameter and 50 cm. in length (6), 1 separatory funnel with a capacity of one liter, and 2 iron supports provided with iron clamps, rubber tubing, and pinch cocks. Arrange the apparatus as shown in Fig. 1.

(c) *Calcium chloride drying tube* (7).—A one-hole rubber stopper and suitable glass and rubber tubing. Connect as in Fig. 1.

(d) *Control tube*.—Consists of a glass vial about 2 cm. in diameter and about 6½ cm. long (8), a 2 hole rubber stopper, and 2 glass tubes. Connect as in Fig. 1.

(e) *Alpha ray electroscope*.—Consists of a head, which is either permanently attached as in Fig. 1 or detachable, as furnished by laboratory supply houses; an open discharge chamber; and an adjustable reading microscope (9) on a permanently attached base. The microscope contains a micrometer scale. The head of the electroscope contains a leaf system including the charging wire (10), and the open discharge chamber contains an adjustable support for the pan (11) and is provided with a door (12).

(f) *Emanation type electroscope*.—Consists of a head, which is either permanently attached as in Fig. 1, or detachable as furnished by laboratory supply houses; a gas-tight emanation chamber (13); and an adjustable reading microscope (14) on a permanently attached base. The microscope contains a micrometer scale. The head of the electroscope contains a leaf system consisting in part of a gold leaf and the charging wire (15). The gas-tight emanation chamber is provided with two stopcocks (16 and 17).

(g) *Charging device*.—The type furnished by laboratory supply houses or consisting of a hard rubber rod and catskin.

(h) *Stop watch*.

(i) *Vacuum pump*.

#### QUALITATIVE DETERMINATION.

(Applicable to solids only)

Charge the leaf system in the alpha ray electroscope through the charging wire (10) to such an extent as to bring the leaf to a suitable position on the scale in the reading microscope (9). Close the door (12) and read the position of the leaf on the scale in the reading microscope five times, at intervals of about 10 minutes, noting the exact time of reading. Calculate the average rate of fall of the leaf in divisions per minute, designating the figure obtained as the natural leak of the instrument for that particular determination.

Place a convenient portion of the sample in solid form on the pan (11) and introduce it into the discharge chamber, close the door, recharge the leaf system, and record the average rate of fall of the leaf in divisions per minute, as before. An increase in the rate of fall of the leaf shows that the sample is radioactive.

#### QUANTITATIVE DETERMINATION.

(Applicable to clear solutions only)

#### PREPARATION OF SAMPLE.

(Description of methods in course of preparation)

#### STANDARDIZATION OF ELECTROSCOPE.

Determine the natural leak of the emanation type electroscope as follows: Create a vacuum in the emanation chamber (13) by means of the vacuum pump. Connect

the closed emanation chamber with the freshly filled calcium chloride drying tube (7) and with the glass vial (8), which should contain about 2 cc. of water. Allow dried air to enter the emanation chamber at the rate of one or two bubbles per second. When equilibrium is established, close off the emanation chamber by turning the stopcock (17), and illuminate the gold-leaf system and the micrometer scale in the reading microscope (14) by placing a small electric bulb a distance of about 10 cm. behind the electroscope. Charge the electroscope through the charging wire (15) to such an extent as to bring the gold leaf in view on a suitable part of the scale. Revolve the charging wire and ground it on the inner wall of the head of the electroscope. When from 15–30 minutes have elapsed, begin recording the exact positions of the gold leaf with respect to the scale, at intervals of about 10 minutes for approximately an hour, noting the exact time and estimating to tenths of divisions. Calculate the average rate of fall of the gold leaf in divisions per minute and designate the figure obtained as the natural leak of the instrument for the particular determination.

Place about 1½ liters of the 2 per cent sodium hydroxide solution (Reagent A) in the 2 liter flask (2) and boil the solution vigorously for at least 10 minutes. Lower the flame somewhat, manipulate the clips so as to fill the gas-collecting buret (5) with the solution, and continue boiling gently. Connect the Erlenmeyer flask containing the standard dilute radium solution (Reagent C) with the buret, after scratching the capillary end of the glass tube with a file. Heat the sample gently and before excessive pressure is generated break off the capillary end inside the rubber connection, noting the exact time of breaking. Open the clip to permit evolution of gas into the buret. Continue heating the sodium hydroxide solution in the 2 liter flask (2) and boil the standard radium solution rather vigorously for 20 minutes. Extinguish the flame under the sample, close the clip so as to retain the boiled-out gases in the buret, and disconnect the Erlenmeyer flask containing the standard radium solution. Before the standard radium solution is cold, draw out and seal off the glass tube of the Erlenmeyer flask containing it. Fill the gas transfer buret (6) with hot 2 per cent sodium hydroxide solution that has been boiled for at least 10 minutes and connect it with the upper end of the gas collecting buret (5), manipulating the clips in such a way as to transfer, without loss, the boiled-out gases from the gas collecting buret (5) to the transfer buret (6). Shut off the burner, disconnect the transfer buret from the gas collecting buret, and connect it with the glass vial (8), the calcium chloride drying tube (7), and the emanation chamber (13), in which a vacuum has been created. Perform this operation without loss of boiled-out gases. Allow these gases to pass into the emanation chamber at the rate of 1–2 bubbles per second. When the sodium hydroxide solution first enters the inlet tube of the glass vial, disconnect the gas transfer buret and allow the inflow of air to continue at the same rate until bubbling ceases. Close the stopcock (17) of the emanation chamber and disconnect the glass vial and the calcium chloride drying tube. Allow the electroscope to stand 2½ hours, charge it, and keep it charged for 15 minutes; then begin a series of readings, recording the rate of fall of gold leaf in divisions per minute in the same manner as in determining the natural leak of the electroscope. Read the scale to tenths of divisions and observe the time of reading with the aid of the stop watch. After the readings have been completed, draw air continuously through the emanation chamber by means of the vacuum pump until the rate of fall of the gold leaf becomes normal. (This operation will require several hours.) Subtract the natural leak of the instrument in terms of divisions per minute from the rate of fall in divisions per minute when the boiled-out gases were contained in the electroscope. Divide the number obtained into the number of millimicrocuries of radon in the standard dilute radium solution (Reagent C). (If the sample has been sealed for less than 30 days, calculate the radon content of the sample from a table of decay or growth of radon. If the sample has been sealed for 30 days or more, the radon

content will be equivalent to the radium content.) The quotient will be the quantity of radium that will cause an acceleration of one division per minute in the rate of fall of the gold leaf. Repeat the standardization occasionally, using other dilute radium solutions prepared from the standard stock radium solution.

#### PROCEDURE.

Determine the natural leak of the electroscope, boil off the emanation from the sample, and determine its effect on the rate of fall of the gold leaf exactly as described previously under "Standardization of Electroscope", taking the precaution of allowing the boiled-out gases to remain in the gas collecting or gas transfer burets at least 10 minutes for decay of any thorium emanation that may be present. Subtract the natural leak in divisions per minute from the increased rate of fall, expressed in divisions per minute, the difference being the rate of fall of the gold leaf due to radon in the sample. Multiply the figure obtained by the number of millimicrograms of radium that will cause an increase of one division per minute. The result will be the quantity of radium in the sample if it has been prepared by boiling and sealing off and has been allowed to stand for at least 30 days. If the sample, after being boiled and sealed off, has stood for less than 30 days, calculate the radium content from tables of decay and growth of radon. If the original sample is a solution, report the content of radium in millimicrograms per cc. or per liter; if the original sample is a solid, report the content of radium in terms of millimicrograms per gram or per 100 grams.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the method described above be adopted as a tentative method.
- (2) That the associate referee for next year (1) develop suitable methods for the preparation of miscellaneous samples for analysis and (2) prepare a description of the preparation of a standard stock solution of radium.

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<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, **8**: 267.



## REPORT ON LAXATIVE AND BITTER TONICS.

By H. C. FULLER (Institute of Industrial Research, Washington, D. C.),  
*Associate Referee.*

The work undertaken during the past year has been on the cascara assay. The method was described in 1922<sup>1</sup>. Certain modifications in the procedure have been made in order to perfect its operation and to eliminate sources of error.

*Collaborative results on cascara sagrada assay.*

COLLABORATOR	TOTAL ANTHRAQUINONES			
	Gravimetric assay	Colorimetric checks		
		Yellow	Red $\frac{1}{8}$ in. cell	Red $\frac{1}{8}$ in. cell
L. Burritt Bureau of Internal Revenue Washington, D. C.	<i>per cent</i> 3.34	9	4.5 R 0.8 Y	Too dark to read
	3.14	8.6	4.2 R 0.6 Y	Too dark to read
	3.15*	1.5		1.6
P. J. Valear Bureau of Internal Revenue Washington, D. C.	2.9	9.0	5.0 R 0.8 Y	Too dark to read
	3.3*	1.5		1.6
E. C. Merrill	3.15			
	3.06			
C. K. Glycart Food and Drug Inspection Station, Chicago, Ill.	2.51	4.25		4.71
	2.54	4.25		4.70
B. L. Murray	2.42	12 Y 1 R	7 R 2.5 Y	
	2.31		6 R 2.5 Y	
E. F. Kenney	2.41			
	2.52			
H. C. Fuller	3.32			
Average . . . . .	2.82			

\* 1 gram sample used.

The principal modification was a preliminary washing, with 2 per cent sodium bicarbonate, of the chloroform solution of the anthraquinones

<sup>1</sup> J. Assoc. Official Agr. Chemists, 1923, 7: 7.

before they were removed from the solvent with sodium hydroxide. While this may be of some advantage in the case of an assay of the crude drug, there is some doubt about its expediency in the case of the fluid-extract.

The results by the gravimetric assay are good, but the colorimetric checks are less uniform than those reported last year<sup>1</sup>.

It will be noted that the average reported this year is practically the same as that shown last year on the same sample, namely 2.80 per cent. It is proposed to offer the gravimetric portion of this assay method to the association for adoption as a tentative method for assaying cascara bark and the fluidextract. The method is as follows:

Use 5 grams of the powdered drug or 5 cc. of the fluidextract, removing the alcohol from the fluidextract by evaporation. Introduce the sample into an Erlenmeyer flask of 500 cc. capacity; add 200 cc. of chloroform and 50 cc. of 25 per cent sulfuric acid, and attach to a reflux condenser (water-cooled), using a cork stopper covered with tin foil. Apply low heat of a Bunsen flame and allow the chloroform to boil for 2½ hours; allow to cool and transfer to a separatory funnel, washing out the flask with a little fresh chloroform.

Draw off the chloroform into another separatory funnel. Add 50 cc. of chloroform to the acid mixture; agitate; and, after separation has taken place, run chloroform into that previously collected. Repeat the procedure three times. Discard the acid mixture.

Collect the chloroform shake-outs in an Erlenmeyer or distilling flask; recover about ¾ of the solvent by distillation, and pour the balance into a separatory funnel, washing thoroughly to remove final traces of anthraquinones; agitate with 25 cc. of 10 per cent sodium hydroxide; draw off the chloroform; and subject to another treatment with sodium hydroxide (10 per cent). Repeat the procedure and finally wash the chloroform with 25 cc. of water.

Unite the alkaline solutions and washings, add an excess of hydrochloric acid, and shake out five times with chloroform. Discard the acid and wash the chloroform with 50 cc. of water (by shaking). Let settle completely, filter the chloroform through cotton in the stem of the funnel into a distilling or Erlenmeyer flask, and recover a portion of the solvent. Then pour the balance into a tared dish, washing out the distilling flask with chloroform, evaporate the solvent, dry at not over 100°C. for 30 minutes, cool in a desiccator, and weigh. The weight represents the total anthraquinone bodies in the drug.

#### NOTES AND COMMENTS.

The colorimetric values obtained by different workers are at such variance that it does not seem wise to recommend this feature at the present time. The experience of the referee indicates that a color check can best be obtained by comparing in Nessler tubes, the shade given by the sample with that given by an alkaline solution of the anthraquinones extracted from a drug, the assay of which has been determined gravimetrically.

The chief sources of error in the assay appear to be overheating in the presence of the sulfuric acid and incomplete removal of the anthraqui-

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8, 23.

nones by chloroform and perhaps by alkali. Sufficient heat should be applied to boil the chloroform, but not the acid.

#### RECOMMENDATIONS.

It is recommended—

(1) That the gravimetric assay be adopted by the association as tentative.

(2) That the colorimetric check be given further study with a view to simplifying and rendering it more accurate.

#### REPORT ON MERCURIALS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The work of the past year has been limited to a collaborative study of two quantitative methods for mercuric chloride in antiseptic tablets, viz., the methods of Rupp and Jamieson.

It has not been demonstrated that the presence of citric acid or ammonium chloride causes any serious difficulty in the quantitative estimation of mercuric chloride by either of the above-named procedures.

The two methods studied are about equal in facility of operation and in the time consumed for a complete analysis. Both lend themselves readily to the routine examination of a large number of samples, and neither requires unusual or expensive apparatus.

The following directions were sent to the collaborators. The outline of the Rupp method is essentially as stated by the author, but it should be expressed in much simpler terms.

#### IODATE METHOD.

(By G. S. Jamieson<sup>1</sup>.)

#### REAGENTS.

(a) *Thiocyanate*.—Dissolve 39 grams of ammonium thiocyanate and 29 grams of zinc sulfate in water and make up to a volume of 1 liter.

(b) *Thiocyanate wash solution*.—Add 10 cc. of the thiocyanate reagent to 490 cc. of water.

(c) *Standard potassium iodate solution*.—Dissolve 19.2191 grams of potassium iodate in water and make up to 1 liter. One cc. of the solution thus prepared is equivalent to 0.00406 gram of mercuric chloride.

#### PROCEDURE.

Weigh 5 grams of the sample, dissolve in water, and dilute to 250 cc. Pipet 10 cc. portions of this solution into 100 cc. beakers. Add 25 cc. of the thiocyanate reagent and dilute to about 75 cc. Vibrate the solutions by striking the sides of the beaker with a glass rod. Allow to stand for 5 minutes, then stir with a glass rod, previously moistened with water. After the mixture has stood for at least one hour, it may be treated gravimetrically or volumetrically, as follows:

<sup>1</sup> *J. Ind. Eng. Chem.*, 1919, 11: 296.

(a) *Gravimetric Method.*

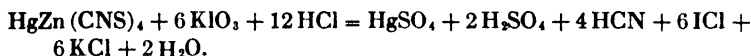
Transfer the precipitate to a tared Gooch crucible, wash four or five times with the thiocyanate wash solution, dry at a temperature between 102° and 108°C., and weigh. Multiply the weight of the precipitate by the factor 0.54493 to obtain the weight of mercuric chloride.

(b) *Volumetric Method.*

Transfer the precipitate to a 7 cm. filter paper that is supported by a platinum cone, using a gentle suction. (In case a platinum cone is not available a double filter paper will be found to serve equally well.) Wash as directed in the gravimetric method. After washing, remove the paper containing the precipitate, fold once, and press lightly between filter or blotting paper to absorb the excess of the thiocyanate solution. Place the paper with the precipitate in an 8 oz. glass stoppered bottle. Add 35 cc. of concentrated hydrochloric acid, 10 cc. of water, and 7 cc. of chloroform. Titrate immediately with the potassium iodate solution, keeping the bottle in constant rotation until the color of the iodine has disappeared from the solution; stopper the bottle; and shake vigorously for about half a minute. Continue the titration with frequent shaking of the bottle until the color of the iodine just disappears from the chloroform layer. Multiply the number of cubic centimeters of potassium iodate solution required by the factor 0.00406, which gives the weight of mercuric chloride.

NOTE: The separation of the crystals is facilitated by striking the outside of the beaker after the solutions are mixed.

The chemical reactions of this method are expressed by the following equations:



The mercury-zinc thiocyanate is slightly soluble in pure water.

**FORMALDEHYDE METHOD.**

(By E. Rupp<sup>1</sup>.)

**REAGENTS.**

- (a) *Potassium iodine solution.*—Dissolve 25 grams of potassium iodide in 50 cc. of water.
- (b) *Sodium hydroxide solution.*—Normal strength.
- (c) *Formaldehyde solution.*—U. S. P. strength, 37 per cent by weight.
- (d) *Acetic acid.*—18 per cent solution.
- (e) *Iodine solution.*—0.1 N.
- (f) *Sodium thiosulfate solution.*—0.1 N.
- (g) *Starch solution.*—1 gram in 200 cc. of water.
- (h) *Gum arabic solution (acacia).*—5 per cent solution in water.

**PROCEDURE.**

Use the same solution as directed in the iodate method.

Pipet a 10 cc. portion of this solution into a 250 cc. Erlenmeyer flask. Add the following solutions: 5 cc. of potassium iodide, 5 cc. of gum arabic, 30 cc. of normal sodium hydroxide, and 3 cc. of formaldehyde. Mix thoroughly and set aside for 10 minutes, with occasional shaking. Acidify with the acetic acid, mix well, and add from a buret 50 cc. of 0.1 N iodine solution. Allow to stand with occasional shaking until the mercury is dissolved. Titrate the excess of iodine with the 0.1 N sodium thiosulfate, using the starch indicator.

One cc. of 0.1 N iodine solution is equivalent to 0.01358 gram of mercuric chloride.

<sup>1</sup> *Ber.*, 1906, 39: 3702; 1907, 40: 3276; *Arch. Pharm.*, 1905, 243: 300.

## NOTES.

In an alkaline medium formaldehyde reduces the mercury in the mercuric chloride to metallic mercury.

The presence of gum arabic solution prevents the agglomeration of the mercury particles and facilitates the solution of the metal by the iodine.

The reaction between the iodine and the mercury is expressed by the following equation:



## COLLABORATIVE RESULTS.

*Percentage of mercuric chloride in antiseptic tablets by the iodate and formaldehyde methods.*

COLLABORATOR	IODATE		FORMALDEHYDE
	Gravimetric	Volumetric	
J. M. Anderson United Drug Co. Boston, Mass.	<i>per cent</i> 44.14	<i>per cent</i> 44.35	<i>per cent</i> 44.47
J. F. Ellis Bureau of Chemistry Washington, D. C.	42.35 <sup>1</sup>	43.46 <sup>2</sup>	43.61 <sup>3</sup>
W. F. Kunke Bureau of Chemistry Washington, D. C.	43.98	44.10	45.37
A. G. Murray Bureau of Chemistry Washington, D. C.	44.2 <sup>4</sup>	44.1	44.2 <sup>5</sup>
G. C. Spencer	43.8 <sup>6</sup>	44.5 <sup>7</sup>	42.7 <sup>8</sup>
T. M. Willgerodt Lehn & Fink New York, N. Y.	44.24 <sup>9</sup>	45.04 <sup>10</sup>	
Averages	43.78	44.25	44.07

<sup>1</sup> Average of seven determinations; <sup>2</sup> Average of five determinations, <sup>3</sup> Average of six determinations, <sup>4</sup> Average of three determinations; <sup>5</sup> Average of four determinations, <sup>6</sup> Average of eight determinations, <sup>7</sup> Average of three determinations; <sup>8</sup> Average of six determinations; <sup>9</sup> Average of six determinations; <sup>10</sup> Average of eleven determinations.

## COLLABORATORS' COMMENTS.

*A. G. Murray.*—For the Rupp method I suggest that 4 per cent sodium hydroxide be specified instead of "normal". Acetic acid, 18 per cent, is an uncommon reagent. Would not the concentrated acid do? Would there be any objection to mixing the acacia and formaldehyde solutions? Is not the pipet as satisfactory as the buret for measuring the iodine solutions? Is the great excess of iodine specified necessary or helpful?

The suggestions of Murray are largely covered by the observation made previously that the Rupp method should be stated in much simpler language.

The great excess of iodine solution called for seems to be necessary to get a complete solution of the metallic mercury.

The gravimetric determination of the mercury by the writer gave 43.64 per cent and 43.82 per cent of mercuric chloride.

#### CONCLUSIONS.

It is evident that the accurate determination of mercuric chloride in antiseptic tablets is yet to be accomplished by the formaldehyde method of Rupp. The writer's experience shows that a wide range of percentages may be obtained by this procedure. The iodate method gives more consistent results, although a close duplication between the gravimetric and volumetric results is still to be attained.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended that further study be made of the formaldehyde method of Rupp and the iodate method of Jamieson for mercuric chloride in antiseptic tablets.

No report on methylene blue was given by the associate referee.

No report on papain was given by the associate referee.

### REPORT ON PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS.

By S. PALKIN (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

Of all the diluents and excipients generally used with phenolphthalein, chocolate has offered the greatest interference in the quantitative determination, because of the large quantity of fat it contains. The preliminary removal of the fat by extraction with petroleum ether (as indicated in a method published by the writer<sup>2</sup>) affords an unsatisfactory solution of this difficulty, as a small quantity of phenolphthalein is apt to be carried down with the fat solution owing to the presence of moisture in the sample, the variability of the composition of petroleum ether, and imperfections in the extraction thimble.

By the introduction of a few additional steps, the "iodination method" (Method I<sup>2</sup>), upon which a report was submitted last year, has been made applicable to chocolate-phenolphthalein preparations.

#### METHOD.

The determination of phenolphthalein in the presence of chocolate or chocolate preparations is based on the following procedure: (1) The iodination of the phenolphthalein is carried out at a temperature that is

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 268.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 14; 1924, 8: 30.

low enough to prevent any appreciable saponification of the fat; (2) after the complete conversion of the phenolphthalein to tetraiodophenolphthalein, the alkaline solution of the latter is filtered in the cold, thus separating it from the solid *fat* that remains behind; (3) any trace of fatty acid present as the potassium salt in the alkaline solution is removed when the *hot acidified* solution is subjected to filtration, and the precipitated tetraiodophenolphthalein and this precipitate are washed with petroleum ether.

The reagents used and the procedure for the determination of the phenolphthalein are essentially as described in the previous report, to which reference has been made.

#### REAGENTS.

(a) *Potassium hydroxide solution*.—Dissolve about 100 grams of potassium hydroxide in an equal weight of water.

(b) *Hydrochloric acid*.—Concentrated.

(c) *Iodine reagent*.—Dissolve 20 grams of potassium iodide in a minimum quantity of water; add 14 grams of iodine and when dissolved dilute to 120 cc.; now add enough strong potassium hydroxide solution to discharge all the iodine.

(d) *Sodium sulfite solution*.—15 per cent.

#### PREPARATION OF ALCOHOLIC EXTRACT.

Introduce a 1 gram sample in a 50 cc. volumetric flask; add about 35 cc. of 95 per cent alcohol; boil gently for about 20 minutes, rotating the flask occasionally; cool and make up to volume with alcohol; mix thoroughly and filter through dry paper, covering the funnel with a watch glass to avoid evaporation; pipet a number of aliquots of 10 cc. each into 250 cc. beakers; and evaporate to dryness on the steam bath to remove the alcohol completely.

#### DETERMINATION.

Take up the residue in alkali by moistening with about 1 cc. of the strong potassium hydroxide reagent and add a little water. When the residue is completely in solution, add a piece of ice, about 40 grams, and pour in the prepared iodine reagent (4-4½ cc.); add concentrated hydrochloric acid from a buret, drop by drop, using a stirring rod (beaker is not rotated) to complete precipitation. Then make alkaline, using drop by drop a strong potassium hydroxide reagent from a buret until solution is effected—except for the small quantity of fatty material that remains undissolved. Repeat this process three or four times. Then add 1 or 2 cc. of sodium-sulfite solution (15 per cent), and filter the whole (ice cold mixture) through a Gooch into a tall 250 cc. beaker, using a bell jar arrangement and washing several times with distilled water. Acidify the filtrate with concentrated hydrochloric acid, using a few cc. in excess, and heat on the steam bath for 20-30 minutes. Filter the coagulated precipitate hot through a weighed Gooch, wash a few times with water, and, when sucked fairly dry, wash several times with petroleum ether. Dry the precipitate in the oven (120°-140°C.) to a constant weight. The weight of the precipitate multiplied by the factor 0.3871 gives the weight of phenolphthalein.

The results obtained by the collaborators, given in Table 1, show a reasonable concordance.

## DISCUSSION.

The accuracy of this revised procedure and the noninterference of comparatively large quantities of cacao fat with the determination of phenolphthalein was demonstrated by the following series of experiments: Additional fat in varying quantities was added to the extracts of the sample of phenolphthalein-cocoa mixture submitted for collaborative analysis, and the phenolphthalein content was determined in each case. For this purpose 1 gram of cocoa in a 50 cc. volumetric flask was treated with alcohol on the steam bath, cooled, and diluted; the flask was filled to the mark with alcohol and filtered. Aliquot portions of 5, 10, and 20 cc. of this extract were added, respectively, to each of the three 10 cc. aliquots of the alcoholic extract obtained from the sample, prepared as described previously in the method under "Preparation of Alcoholic Extract". The combined extract in each case was evaporated to dryness, and the phenolphthalein determinations were made in the usual way described in the method given previously. The results are shown in Table 2.

RECOMMENDATION<sup>1</sup>.

It is recommended that the methods for phenolphthalein tentatively adopted last year and the additional provisions for chocolate-phenolphthalein preparations described in this report be adopted officially.

TABLE 1.

*Percentage of phenolphthalein obtained by collaborators on Sample II\*.*

E O EATON	C. HARRISON	H. R. WATKINS	W. F. KUNKE	S PALKIN
33.58	33.19	33.44	32.98	33.32
33.38	33.16	32.73	33.36	33.17
32.7		32.9	33.13	33.35
..		32.94	33.23	33.5
.	..	33.19		33.47
.		33.12		32.7
...		33.54		.
....	..	32.75		...

\* Sample II was prepared by mixing 1 part of phenolphthalein and 2 parts of cocoa, by weight. Sample I, analyzed by the collaborators last year, did not contain chocolate

TABLE 2.

*Effect of varying quantities of cacao fat on the determination of phenolphthalein.*

EXPERIMENT	EXTRACT, SAMPLE II	ADDED FAT	PERCENTAGE OF PHENOLPHTHALEIN IN SAMPLE II
1	10 cc. A*	5 cc. B†	33.33
2	10 cc. A*	10 cc. B†	33.28
3	10 cc. A*	20 cc. B†	33.55

\* A—Alcoholic extract of 1 gram of Sample II in 50 cc. volume.

† B—Alcoholic extract of 1 gram of cocoa in 50 cc. volume

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 268.



## REPORT ON DIMETHYLAMINOANTIPYRINE (PYRAMIDON)<sup>1</sup>.

By ALFRED W. HANSON (U. S. Food and Drug Inspection Station,  
Chicago, Ill.), *Associate Referee*.

Last year collaborative results were submitted on qualitative and quantitative methods for the determination of pyramidon<sup>2</sup>. It was recommended that four of the qualitative tests be adopted as tentative and that the quantitative methods be studied collaboratively during the present year. The two quantitative methods submitted to collaborators this year were essentially the same as previously submitted, but directions were given not to heat the pyramidon hydrochloride above 100°C.

The following samples were sent to collaborators, and their results are submitted in this report.

### DESCRIPTION OF SAMPLES.

No. 1 consisted of pyramidon.

No. 2 contained 34.94 per cent pyramidon.

No. 3 consisted of a powder obtained by powdering commercial pyramidon tablets. The pyramidon declared was 91.1 per cent.

Reports are submitted by H. C. Fuller, Washington, D. C.; E. H. Velte, H. A. Metz Laboratories, 122 Hudson St., New York; W. F. Kunke, Bureau of Chemistry, Washington, D. C.; M. Garretson, Lehn & Fink, Bloomfield, N. J.; and the associate referee.

### *Collaborative results.*

#### SAMPLE NO. 1.

COLLABORATOR	NO. 1—EXTRACTION METHOD	NO. 2—HYDROCHLOR- IDE METHOD	EXTRACTION OF DRY POWDER WITH HCCl <sub>3</sub>
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
E. H. Velte	99.33	99.19	
	99.50	99.05	
W. F. Kunke	99.75	99.53	
	99.79	99.27	
A. W. Hanson	99.6	99.7	
	99.8	100.8	
H. C. Fuller	98.95		
	99.94		
	100.00		

<sup>1</sup> Presented by V. K. Chesnut.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 29.

## SAMPLE No. 2.

E. H. Velte	30.6	29.45	
	30.2	29.19	
W. F. Kunke	34.66	32.30	
	34.15	31.46	
	33.03	31.09	
	34.31	32.70	
H. C. Fuller	36.44		
	36.32		
	36.66		
M. Garretson	30.00	30.00	31.34
	30.66	31.16	30.06 (Method No. 2)
	30.33	31.78	
	30.70	31.64	
	31.00	31.60	
	32.00	30.70	
	32.16	33.05	
	33.22	33.887	
	35.72	33.987	
A. W. Hanson	34.18	35.1	33.0
	34.0	34.3	

## SAMPLE No. 3.

E. H. Velte	87.75	89.18	88.4
	87.40	88.53	88.7
W. F. Kunke	92.65	92.21	
	92.70	91.95	
	92.10	89.84	
	91.05	87.84	
M. Garretson	86.38	86.48	88.19
	87.06	88.25	88.97
	87.61	88.15	(Method No. 2)
	87.98	88.51	
	88.04	88.94	
	88.26	92.71	
	88.30	93.05	
	88.33	94.66	
	88.37	88.41	
A. W. Hanson	91.2	91.7	92.9
	92.4	89.4	
H. C. Fuller	No. 1		
	89.77		
	89.86		
	90.27		

## COMMENTS BY COLLABORATORS ON QUALITATIVE TESTS.

E. H. Velte reported in detail on these tests last year. Comments by E. O. Eaton, William Rabak, and the associate referee will also be found in last year's report.

W. F. Kunke reports as follows:

Test No. 1—Purplish blue color.

Test No. 2—Purple to violet color fades. After addition of sulfuric acid a reddish brown.

Test No. 3—Purple to violet color. On standing metallic silver deposited.

Test No. 4—Purplish blue does not disappear so readily as in No. 2.

M. Garretson reported that the qualitative tests were satisfactory in all respects.

## COMMENTS BY COLLABORATORS ON QUANTITATIVE METHODS.

*E. H. Velle.*—It is necessary to dry the residues of pyramidon at 100°C. for 5 minute periods, until constant weight is reached. There was no appreciable loss on drying a sample of pure pyramidon under these conditions.

*M. Garretson.*—The methods of themselves are simple enough and should produce better results than we have obtained here. Method No. 2 would be the better of the two if pure pyramidon were to be assayed, since no error would be introduced by chloroform extraction. Method No. 1 is a necessary forerunner of Method No. 2 if the sample is not pure pyramidon.

## DISCUSSION.

The results on the qualitative test for pyramidon are satisfactory.

Some of the quantitative results are satisfactory, while others are too low. It is believed that the low results are due to incomplete extraction of the pyramidon, and it may be advisable to make one or two more extractions with chloroform.

Method No. 2 appears to give satisfactory results on the residues obtained by Method No. 1. It may also be advisable to study the extraction of pyramidon from the dry powder with chloroform. A few results by that method are reported with the collaborative results. One of the collaborators reported that he found it necessary to dry the pyramidon residues to constant weight, and this precaution should be included in the method.

As the results show that more explicit directions should be given, the methods have been rewritten as follows:

*I—Extraction Method.*

Dissolve a quantity of the finely powdered sample estimated to contain from 0.1–0.2 gram of pyramidon in 10 cc. of 1 per cent sodium hydroxide solution. Transfer to a separatory funnel. Extract the pyramidon with chloroform, using 20 cc. for the first extraction and 15 cc. portions for four successive extractions, or continue until the pyramidon is completely extracted. (A test should be made by evaporating an addi-

tional extraction to assure that the pyramidon has been completely extracted.) Transfer the chloroform solution to another separatory funnel. Wash the chloroform with 5 cc. of distilled water. Filter into a tared beaker. Wash the separatory funnels, wash-water, and filter paper with 10 cc. more of chloroform, taking care that no pyramidon adheres to the funnel. Evaporate the combined chloroform extractions on top of a steam bath, using an air current from an electric fan. Heat the solid residue for 5 minutes at 97°–100°C. in a drying oven. Transfer to a desiccator. After cooling, determine the weight of the residue and calculate as pyramidon. (The residue should be heated at 5–10 minute intervals until constant weight is obtained.)

### II—Hydrochloride Method.

Take a quantity of pyramidon (or of the residue obtained by extraction with chloroform, as directed in Method I, estimated to contain 0.1–0.2 gram. Transfer to a tared beaker. Add about 12 cc. of 0.1 *N* hydrochloric acid, or take enough to give a slight excess, and dissolve the pyramidon. (Pyramidon hydrochloric, which is not volatile at 100°C., is formed.) Evaporate to dryness on the steam bath to remove the excess of hydrochloric acid. Add 5 cc. of distilled water and evaporate to dryness again, repeating the process twice to insure complete removal of the excess of hydrochloric acid. Place the residue, which has been brought to dryness on the steam bath, in a steam or hot air drying oven and dry for 1 hour at 100°C. Transfer to a desiccator. Cool, and weigh as pyramidon hydrochloride. Repeat the drying at  $\frac{1}{2}$  hour intervals to a constant weight. Avoid a loss of pyramidon hydrochloride by taking care that the residue is not heated above 100°C. (If the residue is heated to 110°C., a considerable loss occurs.) Multiply the weight found by the factor 0.8638 to obtain the weight of the pyramidon present.

### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the qualitative tests adopted last year as tentative be made official.
- (2) That the quantitative methods for extracting pyramidon, as rewritten in this report, be studied collaboratively.
- (3) That a method for extracting pyramidon directly from the dry powder also be studied.

## REPORT ON THE SEPARATION OF QUININE AND STRYCHNINE<sup>2</sup>.

By F. L. ELLIOTT (U. S. Food and Drug Inspection Station, Boston, Mass.), *Associate Referee*.

An elixir of quinine, strychnine, and iron was sent out to various collaborators for examination by the Bliss method<sup>3</sup>, previously recommended for adoption, and by the Simmonds method<sup>4</sup>. Since minor changes were made in the latter method, the complete instructions submitted to collaborators are given as follows:

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8, 268.

<sup>2</sup> Presented by A. G. Murray.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1921, 4: 416.

<sup>4</sup> *Analyst*, 1914, 39: 81.

*Modified Simmonds Method.*

Make 50 cc. of the sample acid with citric acid and extract with two 15 cc. portions of ether to remove oily material. Make the aqueous solution alkaline with ammonia and extract the mixed alkaloids in the usual way with chloroform and ether.

Evaporate the chloroform and ether in a tared Erlenmeyer flask to dryness on a water bath. Add a little ether and again evaporate to dryness to remove the last traces of chloroform. Dry at 100°C. for 1 hour and weigh to obtain total weight of mixed alkaloids.

Dissolve the alkaloids in 50 cc. of 10 per cent sulfuric acid, add 5 cc. of 4 per cent potassium ferrocyanide drop by drop from a buret, stirring well, and set aside for a few hours or overnight.

Filter the resulting precipitate through a small (7 cm.) filter and wash three times with 3 cc. of 5 per cent sulfuric acid. Reserve the filtrate for the determination of quinine.

Wash the precipitate immediately into a small separator with water, transferring the precipitate remaining in the flask to the separator by shaking about three times with 3 cc. of ammonia and a small quantity of chloroform. Extract the ammoniacal solution of the precipitate in the separator with 20, 10, 10, and 5 cc. portions of chloroform. Collect the chloroform solutions into another separator and extract the alkaloids with 25, 10, 10, and 5 cc. portions of 20 per cent sulfuric acid; repeat the precipitation with potassium ferrocyanide and other operations, as above, until the chloroform extracts are again obtained, reserving the filtrate for determination of quinine. Evaporate the chloroform carefully, adding a little alcohol toward the end to prevent sputtering. Weigh the residue of strychnine after drying it for 1 hour at 100°C.

Combine the two filtrates resulting from the precipitation with potassium ferrocyanide in a separator, make alkaline with ammonia, and extract with a mixture of two parts of chloroform and one part of ether; wash with two 5 cc. portions of water, evaporate to dryness, add a few cc. of ether, and again evaporate to dryness to remove traces of chloroform. Dry at 100°C. and weigh as quinine.

The usual precautions of washing out stems of the separator after each extraction should be taken each time.

The quinine and strychnine residues should be tested for both quinine and strychnine by available qualitative methods.

*Collaborative results.*

(50 cc. of sample used.)

## BLISS METHOD.

COLLABORATOR	QUININE	STRYCHNINE
	gram	gram
A. Stikarofsky United Drug Co. Boston, Mass.	0.7256	0.0179
	0.7214	0.0187
	0.7209	0.0151
C. H. Hickey Food and Drug Inspection Station Boston, Mass.	0.6633	0.0305
	0.6503	0.0320
T. F. Pappe Food and Drug Inspection Station Baltimore, Md.	0.6575	0.028
	0.6585	0.026
F. L. Elliott	0.6673	0.0244
	0.6732	0.0232

H. C. Fuller	0.6784	0.0223
Washington, D. C.	0.6659	0.0172
	0.6567	0.0090
R. V. Pegau	0.6505	0.0425
Food and Drug Inspection Station	0.6725	0.0170
New York, N. Y.		
Theoretical	0.6787	0.0250

## SIMMONDS METHOD.

A. Stikarofsky	0.7214	0.0227
	0.7164	0.0215
C. H. Hickey	0.6535	0.0235
	0.6633	0.0223
C. K. Glycart	0.630	0.0235
Food and Drug Inspection Station	0.650	0.0275
Chicago, Ill.		
F. L. Elliott	0.6676	0.0233
	0.6660	0.0255
R. V. Pegau	0.6475	0.0165
	0.6655	0.015
Theoretical	0.6787	0.0250

## COMMENTS OF COLLABORATORS.

*A. Stikarofsky.*—In the Bliss method it is not necessary to dissolve the mixed alkaloids in chloroform and go to the trouble of boiling it off later. They dissolve readily in 5 per cent sulfuric acid.

The quinine obtained by the Bliss method gives a test for strychnine, and the strychnine separated by this method gives a test for quinine.

The separated alkaloids by the Simmonds method do not give tests for the other constituent. Also, this process is less troublesome and seems to give cleaner residues.

*C. H. Hickey.*—Titrations of the quinine residues in both methods show it to be about 90 per cent pure. Titration of the strychnine residue in the Bliss method shows it to be about 67 per cent pure; that of the Simmonds method about 75 per cent pure strychnine. The Bliss method does not make as clean a separation as the Simmonds method. The strychnine residue appears resinous and dark colored. An experiment with pure strychnine showed that ether used in the same amounts as in the Bliss method will extract about half the strychnine added, although in the presence of quinine only about one-third of the strychnine is apparently lost.

*T. F. Pappe.*—(Method 1). The method does not give complete separation as both alkaloids were found in both fractions, the amount of quinine present in the strychnine fraction probably being slight. This is to be expected, as the amount of ether used to extract the quinine is sufficient to remove considerable of the strychnine.

Suggestions: Dealccoholization of the sample and removal of the oily material after acidification and before extraction of the mixed alkaloids. Solution of the mixed alkaloids in alcohol instead of chloroform to facilitate solution of the strychnine in the sulfuric acid. Drying of the quinine at 120°–130°C. and weighing as anhydrous quinine, as it is difficult to get constant weight in the monohydrated form.

*C. K. Glycart.*—Volumetric checks could be included in the directions. Method 1 was not performed this year.

## Titration of the alkaloidal residues.

	<i>gram per 50 cc.</i>
Quinine.....	0.615 anhydrous 0.590
Strychnine.....	0.023 anhydrous strychnine 0.022

*F. L. Elliott.*—In the Bliss method strychnine is present in quinine residue and quinine in strychnine residue.

The above conditions were not observed in the case of Simmonds method.

Titration of strychnine residues in the Bliss method indicated approximately 70 per cent purity and 85–93 per cent purity in the Simmonds method.

*H. C. Fuller.*—Residues were colored brown. Strychnine residues in all cases showed qualitative test for strychnine. No. 3 was the nearest to pure white.

*R. V. Pegau.*—(Method 1). Quinine gave no positive test for strychnine (using Buchbinder's zinc and ferricyanide test), but the strychnine gave positive test for quinine. Suggest the use of 20, 15, 15, 10, and 5 cc. portions of heavy ether-chloroform mixture for preliminary removal of alkaloids, also the use of small beaker in lieu of dish for evaporation. \* \* \* 35 cc. of ether be used in the first extraction for the removal of oily material on account of solubility of ether in 250 cc. solution.

Above modifications used to obtain second results. The quinine residue in later results gave no test for strychnine and strychnine residues failed to give test for quinine (erythroquin test).

Method 2. Strychnine residues gave no test for quinine, and the quinine residues gave no test for strychnine.

The removal of alcohol before the preliminary extraction with ether to remove the oily material on account of solubility of quinine in alcohol ether solution. \* \* \* Heavy ether chloroform mixture in the amounts mentioned above is recommended for the preliminary removal of alkaloids.

The above modifications were used in obtaining second results.

## DISCUSSION.

The comments of various collaborators indicate that the Bliss method is unsatisfactory. By special tests by one of the collaborators and the writer on 25 mgs. of strychnine, it was found that 12–13 mgs. of strychnine was removed by the seven ether extractions in the case of the Bliss method.

While neither of the methods gives perfect results the Simmonds method appears to give the best results and deserves further study.

RECOMMENDATION<sup>1</sup>.

It is recommended that the Bliss method be not adopted as a tentative or official method, and that the Simmonds method be studied further, together with other available methods.

<sup>1</sup> For the report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 269.

## REPORT ON METHODS FOR THE EXAMINATION OF SILVER PROTEINATES<sup>1</sup>.

By E. O. EATON (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee*.

Last year's report<sup>2</sup> on this subject contains methods for the quantitative determination of total silver and also of silver ions. No collaborative work was done at that time, but results of analysis on four samples were submitted by the associate referee. This year's work incorporates last year's method for total silver, with a slight modification. The methods proposed last year for detection and determination of silver ions have been deleted, and in their place has been inserted a more simple method involving the same titration as the method for total silver. The methods and results of analysis follow:

### METHOD FOR TOTAL SILVER.

Place 1 gram, accurately weighed, in a 500 cc. Kjeldahl flask. Add 15 cc. of concentrated sulfuric acid and then 10 cc. of concentrated nitric acid. Place on a steam bath for a few minutes, with occasional rotation, to insure a homogeneous mixture. Boil to white fumes. Add more nitric acid, boil again to a clear colorless solution, and cool. Add 100 cc. of distilled water and boil until free of nitrogen oxides. Cool, dilute to 300 cc., add 5 cc. of nitric acid and 5 cc. of ferric ammonium sulfate test solution, and titrate with 0.1 *N* potassium sulfocyanate.

Number of cc. of 0.1 *N* potassium sulfocyanate  $\times 0.010788 \times 100$  = percentage by weight of silver.

### METHOD FOR DETECTION AND ESTIMATION OF IONIZABLE SILVER COMPOUNDS.

Weigh a strip of commercial dialyzing tubing (similar to that catalogued by A. H. Thomas under No. 25922, width 55 mm.) about 1 foot long. Wet with distilled water until uniformly pliable. Shake free of adhering water and partially dry by rolling in a clean paper towel. Reweigh while still moist and place in a 250 cc. beaker. (Sheets of dialyzing parchment paper can be used in place of tubing. Fold a square piece of sufficient size over one end of a glass tube, 1 inch  $\times$  4 inches, and secure it in place with a rubber band. This insures a container of the proper size. Dialyzing material should be kept in a humid container to prevent breaking when handled.) Weigh 1 gram of sample and dissolve in 15 cc. of distilled water; transfer to the dialyzing tube, washing beaker with 5 cc. of distilled water; and add the washings to the dialyzing tube. Calculate, and add sufficient distilled water to the beaker to make a total of 100 cc. (This insures 20 cc. in the dialyzing tube and 80 cc. in the beaker.) Adjust the tubing to form a "U" in the beaker, covering with a watch glass, and place in a cool dark closet for 24 hours.

### *Qualitative Test.*

Test a few cc. of the clear colorless solution from the beaker for silver ions by the addition of a few drops of dilute hydrochloric acid and a trace of nitric acid.

<sup>1</sup> Presented by C. K. Glycart.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8. 49.



*Quantitative Method.*

If silver ions are present, remove 50 cc. of the clear colorless solution from the beaker (representing 0.5 gram of sample), dilute to 100 cc., and add 2 cc. of ferric ammonium sulfate and the same quantity of colorless concentrated nitric acid. Titrate with 0.01 *N* potassium sulfocyanate volumetric solution and calculate to percentage by weight of the silver (ionizable):

1 cc. of 0.01 *N* potassium sulfocyanate = 0.0010788 gram of silver.

*Collaborative results on determination of silver.*

COLLABORATOR	SAMPLE No. 1 COLLARGOL		SAMPLE No. 2 ARGYROL		SAMPLE No. 3 PROTARGOL	
	Total	Ionic	Total	Ionic	Total	Ionic
W. F. Kunke*	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
	79.74	none	19.26	none	7.82	1.73
	79.83		19.26		7.77	
S. Palkin*	79.60	practically none	19.3	practically none	7.89	1.66
E. K. Nelson*	79.83	none	19.31	none	7.87	1.81

\* Bureau of Chemistry, Washington, D. C.

## COMMENTS BY COLLABORATORS.

*S. Palkin.*—I would suggest that the ultra refinement of weighing the moist parchment paper be eliminated and that the analyst be cautioned as to the type of parchment paper necessary in order to obtain satisfactory dialysis; also that sheet instead of tubing be recommended with proper description as to manner of folding, etc., inasmuch as the sheet is more readily obtainable and fully as satisfactory.

No comments were received from the other collaborators.

RECOMMENDATIONS<sup>1</sup>.

It is recommended that the methods herein proposed be adopted as tentative methods and that no further work be done on this subject.

## SUPPLEMENTARY NOTE.

Subsequent to the collaborative work herein reported a limited number of experiments were made by the associate referee to show the reaction to phenolphthalein of the clear aqueous dialyzed solution. This work showed that Samples No. 1 and No. 2 were alkaline and that Sample No. 3 was slightly acid. A number of other commercial brands of silver proteinates were tested; those that contained free silver ions were acid and those that did not were alkaline with one exception, in which case the sample was alkaline and in addition contained free silver ions. Further work should be done on this question as it may be of value in connection with results found by potentiometric methods.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8: 269.

# REPORT ON METHODS FOR DETECTION AND DETERMINATION OF ADULTERANTS IN TURPENTINE<sup>1</sup>.

By V. E. GROTLISCH (Leather and Paper Laboratory, Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

The fuming sulfuric acid and the sulfuric-fuming nitric acid methods for detecting and estimating the quantity of mineral oil adulterant in oil of turpentine were again studied this year. Sets of three samples consisting of (1) pure gum spirits of turpentine, (2) turpentine with 3

TABLE 1.

*Results of polymerization of turpentine containing known quantities of mineral oil.*

SAMPLE	ANALYST	FUMING SULFURIC ACID POLYMERIZATION RESIDUE		SULFURIC-FUMING NITRIC ACID RESIDUE	
		Percentage by Volume	Refractive Index at 20°C.	Percentage by Volume	Refractive Index at 20°C.
No. 1—Pure gum turpentine	1	0.6	1.5112	0.0	..
	2	0.8	1.5120	0.2	..
	3	0.8	1.5164	..	..
	4	0.8	..	-0.5	..
	5	0.2	..	0.0	..
	6	0.0	..	..	..
	7	1.8	1.5008	..	..
	8	0.8	1.5+	..	..
	9	0.5	1.510	..	..
	10	1.6	1.501	0.0	..
		1.8	1.530		
No. 2—Containing 3 per cent mineral spirits	1	2.2	1.4621	1.4	1.4276
	2	2.2	1.4588	3.0	..
	3	2.2	1.4584	..	..
	4	3.3	..	1.5	..
	5	1.8	1.4545	2.3	1.4316
	6	1.5	..	..	..
	7	4.32	1.4655	..	..
	8	1.8	1.5	..	..
	9	2.4	1.457	..	..
	10	3.4	1.4710	0.45	1.423
		3.2	1.4715		
No. 3—Containing 8 per cent mineral spirits	1	5.5	1.4428	5.3	1.4278
	2	6.3	1.4410	5.4	..
	3	5.6	1.4387	..	..
	4	6.4	..	5.0	..
	5	4.8	1.4392	5.5	1.4275
	6	3.5	..	..	..
	7	8.0	1.4462	..	..
	8	5.0	1.4392	..	..
	9	5.2	1.443	..	..
	10	7.4	1.4465	2.55	1.428
		7.6	1.4475		

<sup>1</sup> Hereafter the report on turpentine will be run under the heading "Naval Stores" and not under "Drugs".

per cent added mineral spirits, and (3) turpentine containing 8 per cent added mineral spirits were sent to twelve collaborators. Ten reports have been received. The methods for the collaborative work were practically the same as those sent out last year<sup>1</sup>.

The results are shown in Table 1.

On account of the late date when the samples were sent out, and the fact that the sulfuric-fuming nitric acid method is somewhat tedious and complicated, only four reports on this method were received.

With the fuming sulfuric acid method (Method No. 1) the results on the three samples compare fairly well, with one or two exceptions. The results obtained by Analyst No. 7 are somewhat high, especially on Sample 2, and may be due to the fact that the fuming sulfuric acid that was used in this test was slightly below the required strength. This assumption is supported by comparing the refractive indices of the residues. In the case of pure turpentine (Sample No. 1) the reported refractive indices (1.5008 and 1.501) are just barely above the minimum for a pure turpentine residue, which is 1.5. In the case of the adulterated samples, the higher refractive indices would indicate incomplete polymerization of the turpentine.

No explanation can be advanced for the failure of Analyst No. 6 to get any residue at all with pure turpentine, or for his consistently lower results on Samples 2 and 3. He claims to have rechecked his acid and to have followed out the method as described. Analyst No. 8 for some reason got a result too low on Sample No. 2, though his other results compare favorably with those of the other collaborators.

This method is now in general use throughout the country. It is the official method of the U. S. Federal Specifications Board, the American Society for Testing Materials, and the American Railway Association.

The results reported on the sulfuric-fuming nitric acid method are too few to draw any conclusions. This method should be used only when turpentine analyses are made infrequently; then the time required to prepare the standardized fuming sulfuric acid used in Method No. 1 might as well be spent in running Method No. 2. Suggestions have been made for improving this method, which should be further studied. The first steam distillation is apparently unnecessary, since all mineral oil fractions that might be used to adulterate turpentine are volatile with steam and would be recovered in the second steam distillation. Only the heavier lubricating oils would remain undistilled on the first distillation, and the presence of any appreciable quantity of these would be readily shown by the color of the sample and an evaporation test. When the presence of such oils is indicated by other tests, the first distillation should of course be made. Further study of the nitra-

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 18.

tion with fuming nitric acid to destroy the terpene constituents of the distillate from the sulfuric acid treatment is also suggested. The strength and quantity of fuming nitric acid are apparently too great, as some pure mineral oils, which had been treated according to the directions given in the method, were attacked and destroyed to as high an extent as 40 per cent.

The Grotlisch-Smith method for detecting and estimating coal tar oil in turpentine was also studied this year for the first time, six of the collaborators having tried out the method. The sample sent out for study contained 5 per cent of coal tar solvent naphtha. The reported recoveries of oil from 100 cc. of sample varied as follows: none, 0.3 cc., 0.5 cc., 0.8 cc., 1.5 cc., 2.2 cc., and 3.2 cc. The method is a complicated and tedious one, owing to the difficulty of separating the coal tar oils and the terpenes by chemical methods. Familiarity and experience in handling turpentine polymerization products should give better results. The method needs further study.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the fuming sulfuric acid method for mineral oil in turpentine, described as Method I in this report, be adopted as an official method<sup>2</sup>. (Second and final recommendation.)

(2) That the sulfuric-fuming nitric acid method for mineral oil in turpentine be further studied<sup>3</sup>.

(3) That the Grotlisch-Smith method<sup>4</sup> for coal tar oil in turpentine, and any other methods that may appear applicable, be further studied.

#### PRELIMINARY REPORT ON METHODS FOR MOISTURE IN CRUDE DRUGS.

By RUTH G. CAPEN and JOSEPH F. CLEVINGER (Bureau of Chemistry, Washington, D. C.).

Much work has been done on methods for moisture determinations in foods and food products, but very little on such methods for crude drugs, although the moisture content of crude drugs may be important from the view point of its effect upon their quality, and possibly upon their potency.

A preliminary study has been made of the influence upon the results obtained in different types of crude drugs, of humidity, of the presence of volatile constituents, and of the methods used.

These results, as well as those of further study, will be reported later.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1925, 8, 264.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 408.

<sup>3</sup> *Ibid.*, 409.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1921, 13: 791.

## BIO-ASSAY OF DRUGS.

By J. C. MUNCH (Pharmacological Laboratory, Bureau of Chemistry, Washington, D. C.).

The strength of a number of drugs used in medicine must be determined by testing their effects upon animals, as no adequate chemical tests have been produced. The active principle (or principles) may be unknown, or it may not be possible by present methods chemically to separate the physiologically active constituents. The National Convention of 1900, discussing the 8th Revision of the U. S. Pharmacopoeia, directed that "Physiological tests for determining strength should not be introduced by the Committee". The 9th Revision Committee went a step further and recommended " \* \* \* that biological tests or assays, when accurate and reliable, may be admitted". Accordingly, bio-assays for cannabis and for pituitary preparations were included in the text of U. S. P. IX, and optional assay methods for certain other drugs were given in the appendix. Studies of these assay methods during the past ten years have proved the merit of the assay methods recommended. Somewhat amended in some instances, the text of the forthcoming U. S. P. X will contain the optional bio-assay methods of U. S. P. IX as requirements for aconite, cannabis, digitalis, strophanthus, squill, epinephrine, ergot, and pituitary preparations.

The Bureau of Chemistry was asked by the subcommittee whether it would consider furnishing prototype standards for use in standardizing those drugs and preparations that require bio-assay. After considering the matter from various aspects, the Bureau finally decided that it would be feasible to do so and that supplying these standards would lead to uniformity in application of bio-assay methods. It was believed that such action would tend to reduce the variability and to improve the general character and quality of these drugs as marketed, and would materially aid in the enforcement of the food and drugs act. Accordingly, the Bureau is prepared to test, pack, and distribute such prototype standards as the Revision Committee designates to manufacturers and others requesting them. A statement such as the following will be contained in the preface of U. S. P. X:

The introduction of bio-assay methods into the U. S. P. IX, though mostly optional, assisted in the establishment of reliable methods. These have now been made compulsory for a number of important drugs and preparations and in order to facilitate the adoption of these standards and to provide a greater degree of uniformity in the application of these assays, the Bureau of Chemistry of the U. S. Department of Agriculture at Washington have indicated their willingness to supply substances conforming to the new pharmacopoeial standards.

The Bio-assay Subcommittee of the Revision Committee selected the following standards:

- |  |                           |
|--|---------------------------|
| (1) For digitalis, strophanthus, and squill: | Ouabain.                  |
| (2) For cannabis sativa:                     | Fluidextract of cannabis. |
| (3) For ergot:                               | Fluidextract of ergot.    |
| (4) For epinephrine preparations:            | Epinephrine powder.       |
| (5) For pituitary preparations:              | Pituitary powder.         |

The standards and methods of assay set for these preparations in U. S. P. X have recently been published by the Revision Committee; they are essentially the same as those contained in U. S. P. IX. As the procedures specified are not in frequent use by drug chemists, the salient features of the assays may be of interest. In this connection it should be pointed out that careful attention to details is absolutely necessary to insure success in these assays. The use of special physiological apparatus and the special training required to conduct and interpret bio-assay tests may be expected to limit the number of laboratories conducting these assays.

For aconite preparations the method of assay consists in injecting a solution subcutaneously in guinea pigs weighing between 275 and 325 grams each. The standard dose must kill within six hours at least two of every three guinea pigs injected. The lethal doses as established, per gram body weight of guinea pig, are as follows:

Crude drug	0.00004 gram.
Aconitine	0.000000055-65 gram.
Tincture	0.00035-45 cc.

For cannabis preparations the desired result is a certain minimal degree of incoordination produced when the extract or fluidextract in gelatin capsules is given to dogs by mouth. A standard fluidextract of cannabis is necessary in conducting this test; 0.03 cc. of a fluid extract or 0.004 gram of a solid extract should give the same degree of incoordination within a period of one hour as is produced by a dose of 0.03 cc. per kilogram per body weight of the standard fluidextract.

For digitalis, strophanthus, and squill preparations the "one hour" frog method is adopted. Frogs stored at temperatures below 15°C. are held at a temperature of 20°C. overnight, weighed to within  $\frac{1}{2}$  gram, and placed in running water at a temperature of 20°C. Doses of the preparations properly diluted, and dealcoholized when necessary, are injected in the ventral lymph sac. Exactly one hour later the frog is pithed, and the condition of the heart is determined. If a sufficient dose has been given, the heart will be firmly stopped with the ventricle in systole and both auricles in diastole. In case it is found that some of the injected drug has not been absorbed, the animal is discarded. In order to determine the variation of the frogs used assays are made

with ouabain at the time of testing this class of drugs. A definite figure is specified for the minimum systolic dose of ouabain. In case the assay shows a different minimum systolic dose for the frogs used, the values for the digitalis preparations are altered proportionately. The minimum systolic doses as established, per gram of body weight of frog, are as follows:

Ouabain.....	0.0000005 gram.
Tincture of digitalis.....	0.0055-65 cc.
Tincture of strophanthus.....	0.000055-65 cc.
Tincture of squill.....	0.0055-65 cc.

For epinephrine samples the effect upon the blood pressure of dogs is used as an assay method. A 1:100000 solution of epinephrine as hydrochloride in physiological salt solution is injected into the femoral vein of a dog. The rise in blood pressure is graphically measured by a mercury manometer connected with the carotid artery. The same rise in blood pressure is produced as that which follows the injection of a standard epinephrine solution into the same animal.

For ergot preparations the desired effect is a certain degree of bluing in the comb of a single-comb white leghorn cock corresponding in intensity to the bluing produced by the same dose of a standard fluid-extract of ergot. Injections are made deeply into the breast muscles, and the effects are observed within one to one and one-half hours after administration. A standard fluidextract of ergot produces distinct bluing in a dose of 0.5 cc. per kilo of body weight.

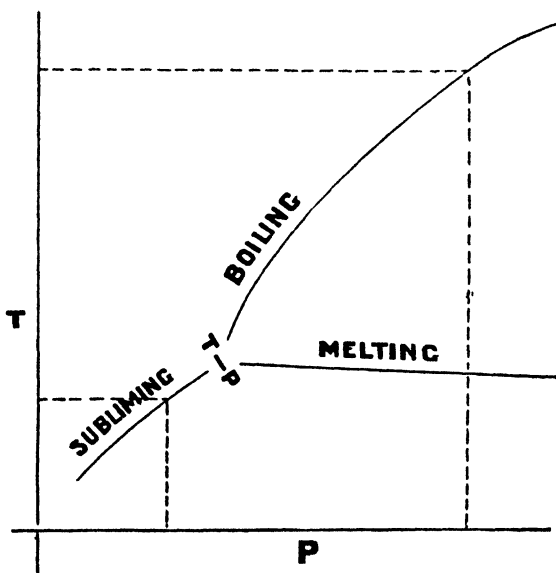
Pituitary solutions are tested by determining the doses that produce the same contraction of an isolated uterus of a guinea pig weighing between 175 and 350 grams as is produced by a solution, 1 cc. of which contains 0.005 grams of a standard, dried, defatted, powdered, posterior pituitary gland. The standard pituitary powder is prepared in accordance with specific directions given in the text of the Pharmacopoeia.

Bio-assays have been made upon a number of these drugs during the past few months. Great variations in the strength of marketed preparations have been found. Many samples have been found to be more than 90 per cent deficient, while others were several times the (optional) physiological strength given in U. S. P. IX. In other instances, all samples examined have been of approximately U. S. P. strength. The present situation with respect to these drugs is worse, perhaps, than with drugs having chemical assay methods, but the official adoption by the U. S. P. X of prescribed bio-assay methods as methods of assay is expected to aid materially in reducing these variations and in improving the quality of these drugs on the market.

## SUBLIMATION AND SOME OF ITS APPLICATIONS.

By JULIUS HORTVET (Minnesota State Dairy and Food Department, St. Paul, Minn.).

An academic discussion of sublimation can not be attempted without a sense of apology, especially to a group of individuals who hold a primary interest in the study of methods applicable in the analysis of drugs. Nevertheless, it seems necessary, in order to emphasize the importance of a number of details presently to be considered, that the phenomenon be elucidated afresh and be stated in an elementary form. A similar undertaking may conceivably be essential as preliminary to a discussion of melting points and boiling points to be determined in connection with analytical procedures. The well-known law expressing the relation between temperature and pressure in connection with boiling has been expressed in extended form as applied to solids, in a manner somewhat as follows: "Every solid substance has, like every liquid substance, at a definite temperature, a definite vapor pressure".



Consulting the diagram it will appear that the curves that represent the conditions for boiling and melting, respectively, of a given substance, intersect at a certain point (T-P), beyond which is a third curve representing the conditions governing sublimation. In other words, a given substance having a melting point practically unchanging, when under a pressure lower than T-P, the so-called triple point, will not undergo



melting but, on the contrary, will change directly from solid to vapor; which is to say, under temperatures and pressures below the triple point definite relationships determine the sublimation of the substance in accordance with the general law already stated. With temperature stationary or variable below the triple point a lowering of pressure may take place to the extent of a practical vacuum with a conceivable improvement in the conditions. A brief tabulation serving to illustrate the application of the above principles may be given as follows:

	MELTING- POINT °C.	TRIPLE- POINT PRESSURE
Water (ice).....	0.0	4.6 mm.
Acetic acid.....	16.4	9.4 mm.
Benzene.....	5.6	35.9 mm.
Iodine.....	114.3	91.0 mm.
Camphor.....	180.0	380.0 mm.
Mercuric chloride.....	288.0	554.0 mm.
Arsenious oxide.....	200.0	1 Atmos.
Carbon dioxide.....	-57.0	5.3 Atmos.

Unfortunately, when it comes to the application of sublimation in a given instance it may likely occur that reference to tables of physical constants will yield information that will be either erroneous or misleading. The general futility of the sublimation temperature figures given in chemical literature may as well be admitted. It is not intended to create the impression that writers or compilers of texts or reference works are addicted to inaccuracy; the trouble lies chiefly in connection with several deficiencies that need to be corrected. Merely to state that a certain substance is sublimable or to specify the temperature under which sublimation takes place does not furnish a complete mental concept that will be useful to the analyst. Presumably, ordinary atmospheric pressure is to be understood in each instance, but from a practical standpoint a statement not only of the pressure conditions but also of the corresponding temperature variables will be more serviceable. A further statement, or elucidation when necessary, regarding the rate or degree of sublimation under given conditions will add further to the usefulness of the information. An extreme case in point illustrating the carelessness (apparently) of compilers may be cited, viz., that of cinnamic acid, the melting point of which is given in one table of reference as 133°C. and in another as -7.5°C. If a preliminary judgment is to be had for the purpose of adjusting conditions for the sublimation of a compound it would seem essential that reliable melting-point figures be available. A number of other examples, although not so glaring, may be cited to emphasize the importance of a thorough, accurate redetermination of these physical constants, accompanied by painstaking observations of the sublimation conditions in all cases. It may be well enough if reliable figures are supplied indicating sublimation temperatures under ordinary atmospheric conditions, but it must be borne in mind that such

figures are seldom serviceable in experimental work, owing, partly, to the serious fact that decomposition often occurs, as in the case, for example, of oxalic acid, which tends to decompose into formic acid and carbon dioxide. In order to be effective, especially in quantitative procedures, and to a scarcely lesser degree in the case of qualitative purifications, it is highly important that a substance be sublimed under conditions that will diminish or entirely prevent not only any tendency toward decomposition but also the equally objectionable liability to change due to contact with atmosphere. Also, there is to be considered the very practical advantage, as well as the increased satisfaction, of conducting sublimations under conditions that facilitate the process and shorten the time.

At ordinary temperature the vapor pressure of a solid substance is, as a rule, extraordinarily small; on heating to a higher temperature the vapor pressure increases. Sublimation is retarded, therefore, owing to the diffusion of accumulated vapor on the surface of the substance. For this reason, at a given temperature, sublimation in a vacuum takes place more rapidly. This signifies an invaluable advantage in connection with the sublimation of substances that will not withstand high temperatures. Obviously, then, a reduction of pressure approaching vacuum accompanied by a gradual though adequate rise in temperature will tend greatly to improve the results. Such will be true especially in connection with so-called microsublimation in which it may be desired to isolate a minute quantity of substance in a comparatively short time.

Prior to the studies conducted by Eder<sup>1</sup> in recent years the advantages of sublimation under vacuum were little appreciated and in fact had scarcely been subjected to practical trial. The work of Eder had chiefly in view the purpose of obtaining characteristic sublimes under suitable conditions for identification. After numerous preliminary trials, experiments were carried out under highly reduced pressures and by means of carefully controlled methods of heating. The substances subjected to study consisted of about 30 alkaloids, classified according to their behavior from the beginning of the first deposits and during the further progress of the sublimation. The following general conclusions are drawn as a result of these investigations:

(a) The tendency toward amorphous forms is diminished, and crystal formation is rendered more definite or rapid the farther sublimation takes place below the melting point of a substance.

(b) In the case of alkaloids with very high vapor pressure the change from an amorphous deposit to crystalline form takes place quickly even when only a small quantity of substance is sublimed.

(c) In the case of alkaloids with medium vapor pressure the majority of substances investigated appear as crystals in a shorter time when the amorphous deposit reaches a certain density.

<sup>1</sup> *Schweiz. Wochschr.*, 1913, 51: 228, 241, 253.

(d) In the case of alkaloids with very low vapor pressure the crystalline forms are amorphous and more or less trifling; crystallization may develop, however, during prolonged sublimation.

(e) Finally there are those alkaloids that commonly occur only in an amorphous condensation.

In the first group occur those alkaloidal bases that exhibit high vapor pressure; in other words, those substances that are directly and easily sublimable from plant tissues. It was demonstrated that a large number of alkaloids, formerly not known to be sublimable, are rendered easily sublimable under vacuum.

The purification of substances by means of sublimation is an operation worthy of more extensive application. Compared with crystallization there is less loss of material, accompanied, in many cases, by a greater removal of impurities. Also, sublimation may be preferred to distillation in special instances, especially when conducted at lower temperatures. There is less risk of decomposition, particularly when the temperature conditions are maintained at the lowest point practicable. Fractional sublimation may be regarded, therefore, as an operation well within practical bounds, and good results are obtainable providing due consideration is given to melting-point conditions and the proper adjustment of temperature and pressure. An excellent illustration of the application of sublimation to crude material is afforded in the separation of cantharidin from the dried insect after suitable preliminary treatment. There is also the well-known separation of caffeine from dried tea leaves or from coffee. Characteristic crystals of ferulic acid are obtainable by subjecting asafetida to sublimation under suitable conditions. Van Itallie<sup>1</sup> has recently described the following distinction which may be made between Sumatra and Siam benzoin: "The Sumatra resin, containing cinnamic acid, forms a sublimate consisting of plates and small rod-like crystals that strongly polarize light; Siam benzoin, containing benzoic acid, gives, under the same conditions, long rod-shaped crystals, which do not strongly polarize light". There may also be cited a number of other illustrations—namely, the separation and identification of santonin in varieties of *artemisia*, the isolation of gentisin from gentian root, and of hydrastine from the hydrastis rhizome. Mention may be made also of the application of sublimation by Wallis as a confirmation of the results obtained in the well-known Reinsch test for arsenic<sup>2</sup>. The properly washed copper spiral, cut into small pieces, if necessary, is packed into the sublimation capsule and covered by means of a slide. By gradually heating the capsule under reduced pressure the arsenic is volatilized and condensed on the slide forming the characteristic octahedral crystals of arsenious oxide. The deposit is in good condition for microscopic exami-

<sup>1</sup> *Pharm. J.*, 1924, 112: 31.

<sup>2</sup> *Ibid.*, 1920, 105: 376; Wallis. *Analytical Microscopy*, 1923, p. 98.

nation without the use of a cover glass and may be further utilized in making microphotographs for purposes of exhibits in forensic cases.

The criticism is well taken to the effect that direct sublimation, applied especially to crude drugs, frequently may not yield results that are perfectly satisfactory or even serviceable. The process may, however, in a number of instances prove valuable as a preliminary operation in order to determine the proper course to pursue in a contemplated analysis by regular chemical methods. It is not intended to create the impression that an unknown mixture may be expected to yield directly, even under vacuum conditions, a desirable result. A preliminary process will be necessary in the majority of instances in order to prepare the material for the separation of the active principle under investigation. The various conditions essential in the sublimator itself have already been indicated, and when to these is added a suitable preliminary preparation of the material one may expect, after a reasonable amount of experience, to obtain some results that will exhibit improvements in comparison with results obtained by well recognized standard methods. Allusion is made chiefly in this connection to quantitative procedures, or so-called assay methods as applied to drugs and pharmaceutical preparations. For example, a question was recently raised in connection with the approved methods applied to ipecac, as to whether sublimation may be serviceable for the separation and identification of the alkaloids naturally occurring in the root. Some of the official assay procedures are admittedly very tedious and long-drawn-out, particularly in connection with the prescribed methods of purification, involving not only loss of material but also the annoying circumstance that a certain quantity of undesirable impurities will inevitably remain in the residue to be subjected to final weighing or identification tests. It is well worth-while, therefore, to consider the possibilities of vacuum sublimation as a means of improving and shortening the present official methods applied to pharmaceutical preparations.

The application of sublimation in connection with quantitative methods of analysis has heretofore received little or no serious attention. The usefulness of the process was recently demonstrated in a forcible manner in connection with the standard method for the determination of vanillin in flavoring extracts. It is well known that the present official method<sup>1</sup> requires a series of fifteen extractions with petroleum ether as a means of removing impurities from the vanillin residue. Skepticism has often arisen regarding the accuracy of the method, especially when the weighing happens to yield vanillin in an unexpectedly small quantity. It may be recalled that Hiltner<sup>2</sup> suggested a modification that consisted in the sublimation, under atmospheric conditions, of the crude residue and determination of the vanillin by loss in weight.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 349.

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull. 152, p. 135.

This modification was discussed by Wichmann<sup>1</sup> in a paper published a few years ago. Some criticisms were pointed out at the time, but no further trials of the method and no modifications were undertaken. Obviously, sublimation under atmospheric conditions, especially by means of direct heating in the manner recommended, may be expected to incur a loss in weight, due not only to the sublimed vanillin but also to evaporation of moisture and other impurities. Two samples of vanilla extract of known origin, made respectively from Bourbon and Mexican beans, were recently carried through the complete official procedure; duplicate samples also were subjected to the same treatment with the exception that the crude residues, after the ether extraction, were subjected to vacuum sublimation in such a manner that the sublimed vanillin could be transferred to a crystallizing dish and directly weighed for the purpose further of checking the results. The sublimation was repeated in each instance. A comparison of the results is given in the following tabulation:

*Comparison of results obtained by the application of various methods  
for determining vanillin in vanilla extract.*

<i>Bourbon Extract</i>	<i>per cent</i>
By official method, A. O. A. C. . . . .	0.113
Crude vanillin residue by official method . . . . .	0.252
By Wichmann's method (under vacuum) . . . . .	0.212
By first sublimation . . . . .	0.210
By second sublimation . . . . .	0.201
<i>Mexican Extract</i>	
By official method, A. O. A. C. . . . .	0.070
Crude vanillin residue by official method . . . . .	0.167
By Wichmann's method (under vacuum) . . . . .	0.162
By first sublimation . . . . .	0.134
By second sublimation . . . . .	0.125

It is apparent from these figures that a very substantial loss of vanillin occurs in the course of the application of the official method, particularly during the purification process. On the other hand, in addition to the greater accuracy in results, the sublimation operation is conveniently carried out and entails no extraordinary care on the part of the analyst. There is also to be pointed out the economy of time.

The official method for benzoic acid prescribes the option either of weighing the purified residue or dissolving in water and titrating with standard alkali. Either of these procedures may be questioned, first, on account of the impurities, which are difficult to remove in certain cases, and, second, on account of the possible presence of an acid substance other than benzoic acid. By direct sublimation of the residue obtained after the first ether extraction there is obtainable a practically pure benzoic acid that may be treated and weighed in the same manner as that described for vanillin. For purposes of comparison, consider the following results obtained on a sample of fruit sirup:

<sup>1</sup> J. Assoc. Official Agr. Chemists, 1921, 4: 479.

Sample labeled "Contains 0.1% Sodium Benzoate".

	AS SODIUM BENZOATE per cent
Sodium benzoate yielded—	
By official A. O. A. C. method . . . . .	0.2064
By subliming of crude residue	
(a) Obtained in official method . . . . .	0.1947
(b) By titration of sublimate . . . . .	0.1943

The present methods for caffeine in coffee and tea entail also a number of uncertainties, as well as inconveniences that may well be eliminated, possibly through the application of vacuum sublimation. By resorting to sublimation on the crude residue remaining after the first chloroform extraction, some satisfactory results have been obtained. A review of the collaborative work on the Power-Chesnut method for caffeine in coffee<sup>1</sup> reveals a variation of 0.25 per cent between the maximum and the minimum results and a maximum variation of 0.17 per cent from the average. On a decaffeinated sample the greatest variation was 0.12 per cent, and the maximum variation from the average was 0.07 per cent. The average of differences between the gravimetric figures and the figures based on the nitrogen results was about 0.10 per cent, the widest variation between the gravimetric and nitrogen results being 0.31 per cent. A sample of coffee yielded the following results when subjected to various methods for the determination of caffeine:

	per cent
Power-Chesnut method . . . . .	1.198
	1.150
Average . . . . .	1.17
Bailey-Andrew method . . . . .	1.30
Sublimate from B-A residue . . . . .	1.22
Nitrogen $\times$ 3.464 . . . . .	1.13

Caffeine sublimes beautifully, not only from the original material but especially from a residue largely freed from impurities. Satisfactory results are obtained by carefully observing certain essential requirements, as follows:

- (1) That the material be distributed in the bottom of the subliming dish in a thin layer, preferably mixed with inert material, as clean sand, magnesia, or carbon.
- (2) That the temperature be gradually increased and carefully controlled.
- (3) That, after apparently complete sublimation, the material be transferred to a crystallizing dish, the solvent evaporated off, and after proper drying of the residue and weighing, the sublimation be repeated until no more sublimate appears to form. In other words,
- (4) It is essential in all cases that the sublimation be prolonged for a sufficient time to insure that the operation is complete.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1921, 5: 267.

A problem has recently arisen in connection with the quantitative estimation of saccharin, particularly in beverages and fruit sirups. Saccharin, which has a melting point of about  $220^{\circ}\text{C}.$ , at which temperature it tends to decompose, has been sublimed under 1-2 mm. pressure at a temperature range of  $104^{\circ}$ - $105^{\circ}\text{C}.$  The sodium compound, however, exhibits certain difficulties that might be anticipated. The problem is to insure complete isolation of saccharin as such, thereby enabling a satisfactory sublimation of the material. A trial was conducted on a sample of strawberry pop, with at first apparently satisfactory results, but on repeating the procedure a lower result was obtained, owing probably to the difficulty referred to. An attempted modification of the official method<sup>1</sup> was undertaken by introducing the sublimation process after the beginning of the fifth line of paragraph 14—in other words, by application of sublimation to the first crude residue. Obviously, a great gain would be effected if it were possible to insure the complete sublimation of the compound, thereby greatly shortening and simplifying the present tedious process.

The foregoing illustrations relate chiefly to methods applicable to the analysis of foods, but on reflection it is recognized that the compounds mentioned occur also more or less prominently in the preparation of drugs. At any rate, the analytical details applicable in one class of products will also be applicable in the other. The same principles are involved, and the same general results are to be attained. Therefore, there exist a common interest in the working out of these problems and a unanimous desire to effect improvements, not only in respect to the accuracy of quantitative as well as qualitative results, but also in respect to the saving of time and labor.

## PRELIMINARY REPORT ON MELTING POINTS.

By JOSEPH F. CLEVENGER (Bureau of Chemistry, Washington, D. C.).

Several interesting phenomena have been observed in determining the melting points of a number of substances by means of a melting-point apparatus that permits a continuous observation, through the microscope, of the material in question. Although it is known that mixtures do not have sharp melting points, it is not believed that the lack of such a point observed in most substances can be explained entirely on the ground that the material contains impurities.

A preliminary study of the melting points of caffeine, anthracene, amygdalin, chinconine, heroin, and cumarin has been made, and a further study is now being made of these and other substances, the results of which will be reported in a later publication.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 121.

## CONTRIBUTED PAPERS.

POST-MORTEM DISAPPEARANCE OF GLYCOGEN AS A  
POSSIBLE INDEX TO SPOILAGE IN CLAMS<sup>1</sup>.

By D. B. DILL (U. S. Food and Drug Inspection Station,  
San Francisco, Calif.).

Disappearance of tissue glycogen is a familiar post-mortem change. Kickton and Murdfield<sup>2</sup> investigated the difference in glycogen contents of beef and of horse flesh and found the method of identification based on this difference reliable only when applied to flesh from recently slaughtered animals. Schöndorff and Wachholder<sup>3</sup> found from none to 0.68 per cent glycogen in fresh-water fish muscle. They found that the glycogen decreased gradually in dead tissue, but that it had not entirely disappeared when the flesh started to decay. Moscati<sup>4</sup> found that human muscles have an average glycogen content of 0.4 per cent; in the muscle of an amputated limb held at 15°C. it diminished slowly and continuously until the 69th hour, when it began to disappear more rapidly. It had completely disappeared 100 hours after death.

## PURPOSE OF INVESTIGATION.

The occurrence and post-mortem disappearance of glycogen in certain species of Puget Sound mollusks is the subject of this investigation. It was undertaken with the possibility in mind of arriving at a satisfactory chemical method of detecting spoilage in canned mollusks. Two possible modifications of the A. O. A. C. tentative method for the determination of sugar in meats<sup>5</sup> were studied.

Three sets of samples were obtained: the first in April, 1922; the second in April, 1923; and the last in June, 1923. In all cases the entire shell contents was employed for analysis. A composite sample including 20 or more individuals was passed through a meat chopper rapidly; suitable samples were removed for sugar and glycogen determinations, and the remainder was held for further analysis. Solids were determined by heating a 5 gram sample to constant weight at about 98°C. and atmospheric pressure. Sugar and glycogen results were then calculated to the dry basis.

## METHODS USED.

The A. O. A. C. tentative method for the determination of sugar in meat is unsatisfactory. The preliminary boiling with water requires con-

<sup>1</sup> From the U. S. Food and Drug Inspection Station, Seattle, Wash. This investigation was carried on under the direction of A. W. Hansen. Valuable criticisms were offered by C. L. Alaberg.

<sup>2</sup> *Z. Nahr. Genussm.*, 1907, 14: 501-11.

<sup>3</sup> *Arch. ges. Physiol.* (Pflüger's), 1914, 157: 147-64.

<sup>4</sup> *Beitr. Chem. Physiol.* (Hofmeisters), 1907, 10: 337-44.

<sup>5</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 242.



stant attention; filtration is exceedingly tedious; and most of the glycogen will be extracted and included with the sugar. On account of these difficulties a method similar in principle to that of Meyerhof<sup>1</sup> was adopted. It was found that by substituting 65-70 per cent alcohol for water as a sugar solvent, the sample can be boiled on the steam bath without bumping. Filtration through a folded filter is very rapid, and a clear filtrate is obtained. Glycogen is not extracted. The following procedure was applied to sugar-free fish flesh after the addition of known quantities of dextrose and was found to give 90-95 per cent recovery:

Twenty-five grams of the ground sample is added to about 150 cc. of 85 per cent alcohol. The sample may then be held until convenient to proceed. After heating to boiling on the steam bath, the alcohol is decanted onto a folded filter and collected in a 750 cc. Erlenmeyer flask. Three subsequent extractions are made with 100 cc. portions of 65 per cent alcohol, after being heated to boiling on the steam bath each time. The collected filtrates are evaporated or may be distilled to recover the alcohol. When a volume of 25-40 cc. has been reached, the resulting aqueous sugar solution is transferred to a 100 cc. volumetric flask and precipitated with phosphotungstic acid as in the A. O. A. C. method. Since reducing sugars only were being considered, inversion was omitted, and after filtration and removal of the excess phosphotungstic acid with dry potassium chloride, sugar was determined directly by copper reduction and determination of the cuprous oxide by the volumetric thio-sulfate method.

In the samples of June, 1923, sugar was determined by the Folin-Wu method<sup>2</sup> for sugar in blood. Preliminary investigation indicated 95-100 per cent recovery and good agreement with this method. Satisfactory duplicates were always obtained. Ten grams of the finely ground sample was used, and the Folin-Wu method was then followed in exact detail.

Glycogen was determined by the A. O. A. C. tentative method<sup>3</sup>. The reprecipitated glycogen was dissolved in water and hydrolyzed with hydrochloric acid, and the reducing sugar was determined as stated previously.

#### RESULTS OF ANALYSIS.

In Table 1 will be found the results of an experiment in which minced fresh clams were placed in the refrigerator and held for 48 hours. Sugar and glycogen determinations were made at frequent intervals.

A similar experiment was carried out in which the whole shucked clams were placed in the refrigerator and held for the same length of time. After 24 hours some of the shucked clams were ground, and sugar,

<sup>1</sup> *Arch. ges. Physiol.* (Pflüger's), 1920, 185: 11-32.

<sup>2</sup> *J. Biol. Chem.*, 1920, 41: 367-74.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 241.

glycogen, and solids were determined. After 48 hours the remainder of the sample was ground, and the same determinations were made. These results are shown in Table 2.

One sample of the freshly shucked clams was minced, ground, and treated with 1 per cent of toluene. Sugar, glycogen, and solids were determined as above. These results are shown in Table 3.

#### DISCUSSION.

Consideration of Table 1 leads to the conclusion that seasonal variation in glycogen content is of the same order of magnitude as the decrease in the percentage of this constituent that occurs during spoilage. Thus the fresh sample of *Paphia staminea* obtained in April, 1922, had a glycogen content of 12.50 per cent (dry basis). When the sample had become putrid the glycogen content had decreased to 3.64 per cent. The fresh sample obtained in June, 1923, had only 4.49 per cent glycogen (dry basis). It is evident that the possibility of detecting spoilage in mollusks by this determination is remote.

The sugar content increased rapidly to a maximum before spoilage took place in minced fresh clams. It will be noted that for each series the sum of glycogen and sugar is approximately constant until spoilage begins.

When whole shucked clams were held at 5°-10°C. (Table 2), spoilage was delayed, but no other difference in the character of the results was noted.

When minced clams were treated with 1 per cent of toluene and held in the refrigerator quite different results were obtained. As shown in Table 3, the glycogen had nearly disappeared within 24 hours and had completely disappeared after 48 hours. Only about one-fourth of the glycogen appeared as sugar.

In spite of the fact that this minced tissue was held below 10°C. and had been treated with toluene, there is a possibility that bacterial contamination was a factor in this disappearance of carbohydrate. However, even after 48 hours there was no odor of bacterial decomposition products and no evidence of gas formation in the tightly stoppered flask. It is also to be noted that even in unpreserved minced clams held for 24 hours under the same conditions, the odor did not indicate advanced decomposition. These facts render improbable an explanation of this experiment involving destruction of carbohydrate by bacteria.

The conversion of carbohydrate to lactic acid in minced muscle has been studied by Meyerhof<sup>1</sup>. The carbohydrate of minced frog muscle held anaerobically at 14°-16°C. in phosphate buffer solution underwent within a few hours almost complete transformation to lactic acid. His

<sup>1</sup> *Arch. ges. Physiol.* (Pflüger's), 1921, 188: 114-60.

TABLE 1.  
Changing sugar and glycogen contents of fresh clams after mincing. Holding temperature, 5°-10°C.

NO.	SPECIES*	DATE	HOLDING PERIOD AND CONDITION	SUGAR (DRY BASIS) per cent	GLYCOGEN (DRY BASIS) per cent
1	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . .	April 1, 1922	Fresh	2.95	12.50
2	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	April 2, 1922	24 hours after grinding (stale)	6.82	8.86
3	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	April 3, 1922	48 hours after grinding (putrid)	6.82	3.64
4	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 16, 1923	Fresh	1.39	4.49
5	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 16, 1923	4 hours after grinding (normal odor)	3.10	2.56
6	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 17, 1923	24 hours after grinding (stale)	2.35	2.67
7	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 18, 1923	48 hours after grinding (putrid)	1.82	0.53
8	Soft-shell clam ( <i>Mya arenaria</i> ) . . . . .	April 1, 1922	Fresh	2.49	10.11
9	Soft-shell clam ( <i>Mya arenaria</i> ) . . . . .	April 1, 1922	6 hours after grinding (normal odor)	5.94	7.79
10	Soft-shell clam ( <i>Mya arenaria</i> ) . . . . .	April 2, 1922	24 hours after grinding (stale)	4.40	5.84
11	Soft-shell clam ( <i>Mya arenaria</i> ) . . . . .	April 3, 1922	48 hours after grinding (putrid)	4.40	2.32

\* The common names employed in all the tables are in accord with those used by F. W. Weymouth, Fish Bulletin No. 4, State of California Fish and Game Commission.

TABLE 2.  
Changing sugar and glycogen contents of whole clams after shucking. Holding temperature, 5°-10°C.

NO.	SPECIES	DATE	HOLDING PERIOD AND CONDITION	SUGAR (DRY BASIS)	GLYCOGEN (DRY BASIS)
1	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . .	June 16, 1923	Fresh	per cent 1.39	per cent 4.49
2	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 17, 1923	24 hours after shucking (normal odor)	2.35	2.25
3	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	June 18, 1923	48 hours after shucking (stale)	2.35	1.50

TABLE 3.  
Changing sugar and glycogen contents of clams after mincing and addition of 1 per cent of toluene.  
Holding temperature, 5°-10°C.

NO.	SPECIES	DATE	HOLDING PERIOD AND CONDITION	SUGAR (DRY BASIS)	GLYCOGEN (DRY BASIS)
1	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . .	April 6, 1923	Fresh	per cent 1.40	per cent 14.10
2	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . .	April 7, 1923	24 hours after grinding (normal odor)	4.80	0.80
3	Rock Cockle or Little Neck clam ( <i>Paphia staminea</i> ) . . . .	April 8, 1923	48 hours after grinding (normal odor)	4.90	None

study was based on living tissue, and no investigation was made of the autolytic processes that follow the "Abtötung" of the cells.

There is not a complete analogy between this investigation and the work of Meyerhof. In this study the entire clams (including viscera) were minced. No buffer substances were added. Molluscan tissue differs in make-up from amphibian tissue. It is quite possible that the plasmolytic power of toluene modified the course of the autolysis. Thus Giglioli<sup>1</sup> has shown that treatment of yeast cells with an anaesthetic such as chloroform permits the escape from the cell of the intracellular enzymes. Also, Armstrong and Armstrong<sup>2</sup> have pointed out that substances having little chemical activity such as toluene, carbon bisulfide, and chloroform "are most active in initiating enzymic change in the leaf". Further, Vinson<sup>3</sup> has shown that in the ripening of dates "any substance which will penetrate the cuticle and kill or stimulate the protoplasm, thereby releasing the previously insoluble intracellular enzymes without rendering them inactive, will bring about ripening". Among the most efficient substances used were ether, chloroform, toluene, and ethylene dichloride.

#### CONCLUSION.

The possibility of detecting spoilage in clams by determination of the post-mortem disappearance of glycogen is remote. While the single experiment on the autolysis of minced clams in the presence of toluene is far from conclusive, it suggests that molluscan tissue, with its high carbohydrate content, is well adapted for the study of carbohydrate metabolism.

### STUDIES IN THE ANALYTICAL CHEMISTRY OF DRUGS<sup>4</sup>.

#### II. MODIFIED PROCEDURE FOR THE ASSAY OF ALKALOIDAL TABLETS.

By E. O. EATON and A. G. MURRAY (Bureau of Chemistry, U. S. Department of Agriculture).

One of the writers (E. O. E.), having occasion to assay some tablets of apomorphine hydrochloride, found it necessary to use a method that would avoid application of heat and undue exposure to air.

Schmidt<sup>5</sup> has described a procedure for the assay of stramonium, hyoscyamus, and belladonna which consists of the following steps: (1) The powdered drug is shaken with a mixture of ether, chloroform, and sodium hydroxide solution; (2) an aliquot of the filtered immiscible solvent is extracted with a measured quantity of standard acid; (3) the excess acid is titrated with standard alkali. Similar procedures

<sup>1</sup> *Atti accad. Lincei*, Rome, [5], 1911, 20, II: 349-61.

<sup>2</sup> *Proc. Roy. Soc. (London)*, Series B, 1910, 82: 588-602.

<sup>3</sup> *J. Am. Chem. Soc.*, 1910, 32: 208-12.

<sup>4</sup> *J. Am. Pharm. Assoc.*, 1924, 13: 691.

<sup>5</sup> *Apoth. Ztg.*, 1900, 15: 13.

have been used by Frerichs and de Fuentes Tapis<sup>1</sup> for the assay of ipecac, by Beckurts<sup>2</sup> for the assay of physostigma, and by Rippetoe<sup>3</sup> for the assay of fluidextract of gelsemium.

The method proposed in this paper involves the same principles. The essential difference from the usual procedure is in the final recovery of alkaloid from the organic solvent. This is accomplished, not by evaporation of the solvent, but by extraction of the alkaloid with a measured volume of standard acid, as thereby the use of heat and undue exposure to air are avoided. As the procedure gives accurate results and is also simple and rapid, it was applied to tablets of various alkaloidal salts; with a few exceptions it gave reliable results. The method is not at present recommended for atropine, morphine, strychnine, or sparteine. Its chief usefulness is, of course, with those alkaloids that are volatile or are easily decomposed by heat or exposure to air. The alkaloids for which it has been tested and found reliable are listed in the last paragraph.

There is necessarily some choice of immiscible solvents and of alkalis used for liberating the alkaloids from their salts. The use of ammonia for liberating the alkaloids is objectionable because of the solubility of gaseous ammonia in the immiscible solvent. Where they are suitable sodium bicarbonate and sodium carbonate are preferred as they are insoluble in the immiscible solvents used and are easily washed away. Calcium or barium hydroxide may be used if necessary to separate phenolic from non-phenolic alkaloids. Any suitable immiscible solvent, whether lighter or heavier than water, may be used. In most of the experiments the writers used a mixture of chloroform and ether (1:3), as this is regarded as the most generally useful solvent. Ether, benzol, or chloroform, however, may be used when suitable for the alkaloid to be extracted.

#### PROCEDURE.

The details of the procedure when the light chloroform-ether mixture is used as the immiscible solvent, are as follows:

A number of tablets containing about 65 mg. of the alkaloid or its salt are dissolved in a separatory funnel in as small a volume of water as practicable. An excess of sodium bicarbonate or sodium carbonate is added, a suitable quantity (25 ml) of the solvent is introduced, and the mixture is shaken. After the two layers have completely separated, the lower layer is withdrawn into a second separatory funnel and extracted repeatedly with small portions (5 ml) of fresh solvent until the alkaloid has been completely removed. The combined solvent is washed three times with small volumes of water, which are collected and extracted with a little fresh solvent. The aqueous portion is discarded, and the solvent is washed once with water and added to the main portion.

To the solution of alkaloid in the separatory funnel is added a measured excessive volume of 0.02 *N* sulfuric acid; the mixture is shaken thoroughly, and the aqueous layer is drawn off into a flask or beaker. The solvent is washed twice with small portions of

<sup>1</sup> *Arch. Pharm.*, 1902, 240: 401.

<sup>2</sup> *Apoth. Ztg.*, 1905, 20: 670.

<sup>3</sup> *Proc. Am. Pharm. Assoc.*, 1910, 58: 1061.

water, which are added to the acid liquid. Finally the excess acid is titrated with 0.02 *N* sodium hydroxide.

If the immiscible solvent chosen is heavier than water, the necessary modifications of the procedure will readily suggest themselves to the analyst.

Illustrative of the results obtained are the following:

(a) A commercial sample of apomorphine hydrochloride—

WEIGHT OF SAMPLE <i>mg.</i>	APOMORPHINE HCl FOUND <i>mg.</i>	<i>per cent</i>
150.3	147.5	98.1
150.7	148.7	98.7

(b) A commercial sample of cocaine hydrochloride—

WEIGHT OF SAMPLE <i>mg.</i>	COCAINE HCl FOUND <i>mg.</i>	<i>per cent</i>
152.8	150.3	98.4

The titrated solution was reextracted and retitrated by the same process. Cocaine HCl found 148.4 mg. (97.1 per cent).

Sodium bicarbonate is preferred as the alkali for liberating apomorphine, cocaine, or diacetylmorphine to avoid danger of hydrolysis. Sodium carbonate is satisfactory for liberating codeine, emetine, homatropine, hyoscyne, hyoscyamine, physostigmine, procaine, or quinine.

## THE PREPARATION OF BUTTER SAMPLES FOR ANALYSIS.

By LLOYD C. MITCHELL and SAMUEL ALFEND (St. Louis Station, Bureau of Chemistry, U. S. Department of Agriculture).

### INTRODUCTION.

The method of the Association of Official Agricultural Chemists for the preparation of butter samples for analysis<sup>1</sup> is receiving considerable criticism. For some time dissatisfaction has existed among food chemists on account of the wide discrepancies often found in the analytical results obtained by different chemists on the same lot of butter and even in check results by the same analyst. It is known that many, perhaps the majority, of dairy chemists and buttermakers do not use the official method, claiming that it yields poor checks and is impracticable.

This paper presents a critical study of the present official method for the preparation of butter samples for analysis, together with data on a widely used commercial method and a proposed method of preparation.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 232. The association, in its new Book of Methods, 1925, published after the completion of the work reported here, revised the official method to read as follows: Soften the entire sample in a closed vessel at as low a temperature as possible. Shake or mix vigorously until a perfectly homogeneous, semi-solid mass is obtained. Weigh the portions for analysis at once. If the sample is kept for any length of time, it must be softened and mixed until semi-solid before portions are withdrawn for analysis.

## OFFICIAL METHOD.

The first record of the method for the preparation of butter for analysis was made by E. H. Jenkins<sup>1</sup>, Referee on Dairy Products for the association in 1890, in preparing a sample of butter for cooperative work. The details of his procedure, which was adopted as official, are as follows:

If large quantities of butter are to be sampled, a butter trier or sampler may be used. The portions thus drawn are to be perfectly melted in a closed vessel at as low a heat as possible, and when melted the whole is to be shaken violently for some minutes till the mass is homogeneous. A portion is then poured into a vessel, from which it is to be weighed out for analysis and should nearly or quite fill it. This sample should be kept in a cold place till analyzed.

TABLE 1.

*Results obtained by melting the butter, shaking violently under a stream of cold water until solidified, and taking duplicate portions from opposite sides of the container.*

SAMPLE NO	MOISTURE <i>per cent</i>	SOLIDS NOT FAT <i>per cent</i>	FAT (BY DIFFERENCE)
			<i>per cent</i>
1	16.22	4.66	79.12
	16.21	4.54	79.25
2	16.30	4.56	79.14
	16.26	4.65	79.09
3	16.16	4.56	79.28
	16.43	4.66	78.91
4	16.13	4.52	79.35
	15.72	4.38	79.90
5	16.19	4.80	79.01
	15.39	4.56	80.05
6	15.60	4.08	80.32
	16.30	4.30	79.40
7	15.23	4.60	80.17
	15.67	4.60	79.73
8	15.66	3.94	80.40
	15.82	3.89	80.29
9	15.79	4.03	80.18
	15.79	4.11	80.11

## Variation among duplicates:

Maximum	0.80	0.24	1.04
Minimum	0.00	0.00	0.05
Average	0.31	0.11	0.41

The results of eleven analysts on this sample varied 0.38 per cent for moisture, 1.07 per cent for casein, 1.88 per cent for fat, and 0.53 per cent for ash. Discussing the moisture determination, Jenkins stated, "It appears to your reporter doubtful whether, if the method and manipula-

<sup>1</sup> U. S. Dept. Agr. Bur. Chem. Bull. 28, p. 48.



tion were faultless, results could be expected to agree much more closely than those herewith reported, considering the difficulty of preparing a sample for analysis which shall be perfectly homogeneous. The results are sufficiently close for any *present*<sup>1</sup> demand".

The drawing off of a smaller sample for analysis, intended primarily to provide a number of samples for collaborative work, appears altogether unnecessary in the preparation of a single sample for analysis.

In comparing the method adopted in 1890 with the official method, as published in 1920, it is noted that they differ only in that the present method gives instructions to cool the sample while shaking until it is

TABLE 2.  
*Results on single sample of "sweet" butter prepared as for the determinations in Table 1.*

LAYER	MOISTURE <i>per cent</i>	SOLIDS NOT FAT <i>per cent</i>	FAT (BY DIFFERENCE) <i>per cent</i>
Upper	13.31		
	13.28		
	13.10		
	13.28		
	13.40		
	13.45	1.01	85.54
	13.35	0.97	85.68
	13.25	0.97	85.78
	13.20	0.98	85.82
	13.42	0.98	85.60
Variation	0.35	0.04	0.28
Middle	13.30		
	13.23		
	13.12		
	13.27		
	13.44		
	13.20	1.01	85.79
	13.21	0.99	85.80
	13.12	1.00	85.88
	13.06	1.01	85.93
	13.42	1.03	85.55
Variation	0.38	0.04	0.38
Lower	13.14		
	13.16		
	13.23		
	13.06		
	13.10		
	13.02	1.01	85.97
	12.92	0.97	86.11
	13.02	1.04	85.94
	13.02	1.05	85.93
Variation	0.31	0.08	0.18
Maximum	13.45	1.05	86.11
Minimum	12.92	0.97	85.54
Average	13.21	1.00	85.81
Total variation	0.53	0.08	0.57

<sup>1</sup> The italics are the writers'.

solidified sufficiently to prevent the separation of the water and fat. This addition was first inserted into the method by the Committee on Revision of Methods and adopted by the association in 1907<sup>1</sup>.

The method of cooling is not specified. This is frequently done by shaking the melted sample violently under a stream of cold water until solidified; or by alternately shaking violently and rotating the container in a pan of ice water until solidified; or perhaps by allowing the sample to cool slowly at room temperature, or in a refrigerator, with occasional violent shaking until it is solidified.

The work which follows shows the unreliability of the official method, and the effect produced by variations in the method of cooling.

TABLE 3.  
*Results obtained by melting the sample, allowing to stand, and shaking violently in the air until solidified.*

LAYER	MOISTURE	SOLIDS NOT FAT	FAT (BY DIFFERENCE)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Upper	15.55		
	15.49		
	15.55		
	15.53		
	15.54	2.99	81.47
	15.52	2.96	81.52
Variation	0.06	0.03	0.05
Middle	15.51		
	15.59		
	15.62		
	15.65		
	15.73	3.02	81.25
	15.68	2.97	81.35
Variation	0.22	0.05	0.10
Lower	15.64		
	15.55		
	15.58		
	15.53		
	15.64	3.02	81.34
Variation	0.11		
Maximum	15.73	3.02	81.52
Minimum	15.49	2.96	81.25
Average	15.58	2.99	81.39
Total variation	0.24	0.06	0.27

#### EXPERIMENTAL.

Table 1 gives the results obtained on samples prepared by melting the butter, shaking violently under a stream of cold water until it has solidified, then taking duplicate portions from opposite sides of the container.

<sup>1</sup> U. S. Dept. Agr. Bur. Chem. Bull. 107, p. 123.

Table 2 shows the results found on a single sample of "sweet" butter prepared as above. Ten samples were taken from various portions of the upper surface; about one-third of the butter was removed, and ten samples were taken from the fresh surface; half of the remaining butter was then removed, and nine samples were taken from the bottom of the container.

In Table 3 are given values obtained on a sample of butter cooled in a different manner. A pound of butter was entirely melted, allowed to stand in the room until a slight cloudiness in the fatty layer appeared, and then violently shaken in the air until solidified. Six, six, and five samples, respectively, were taken from the upper, middle, and lower portions of the sample in the manner shown above.

It is apparent that the method of cooling employed for the samples shown in Tables 1 and 2 gives greater variation than is found in the method used for the sample in Table 3. The results show that different analysts, though following the official method strictly, may differ slightly in their procedure, and that these differences are sufficient to affect considerably the homogeneity of the prepared sample.

Another illustration of the effect of possible differences in the method of preparation is given in the following experiment, in which three samples of butter were prepared by the official method: Sample No. 1 was melted and shaken violently under running water until it reached a temperature of 28.5°C., and from it three samples were taken, two from opposite sides and the third from the center; Sample No. 2 was melted and shaken continuously, first under running water, then in ice water until it reached a temperature of 24°C., and from it three samples were weighed out as before; the third sample was melted, then alternately shaken violently and rotated in ice water until it reached a temperature of 16°C., and from it three samples were taken from the sides of the container at equal distances from each other. (See Table 4.)

TABLE 4.

*Moisture results from three samples prepared under varying conditions.*

	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	12.71	13.91	14.09
	12.48	13.82	14.56
	12.78	13.79	13.72
Variation	0.30	0.12	0.84

From the results in Table 4, it is seen that a difference in the temperature to which the sample is cooled and the rapidity of cooling may affect its homogeneity considerably.

In determinations varying as widely as those reported above, it is desirable to know the actual composition of the butter under examination. A number of known samples were therefore prepared from butter

fat, water, salt, and dried powdered skimmed milk (defatted). These are here designated as "synthetic" butters.

A synthetic butter containing 14.36 per cent moisture was melted and then prepared by alternately shaking and cooling in a pan of ice water until it solidified. Three samples from different parts of the container gave moisture values of 14.80, 14.29, and 14.18 per cent, respectively. Three more samples taken as closely as possible to the first ones gave results of 14.86, 14.48, and 14.09 per cent, respectively. The average moisture value was 14.45 per cent, as against the theoretical 14.36, but the variation was 0.77 per cent.

Another synthetic butter containing 15.65 per cent water was prepared by shaking the melted sample violently in the air until the product solidified. After allowing to stand overnight in the room, five samples were taken from the top, middle, and bottom of the butter, respectively. The moisture results are given in Table 5.

TABLE 5.  
*Moisture results showing homogeneity of sample of known composition, prepared by official method.*

	UPPER LAYER	MIDDLE LAYER	LOWER LAYER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	15.68	15.76	15.69
	lost	15.75	15.43
	15.25	15.67	15.64
	15.43	15.69	15.58
	15.74	15.86	15.77
Variation	0.49	0.19	0.34
Maximum		15.86	
Minimum		15.25	
Average		15.64	
Total variation		0.61	
Theoretical		15.65	

The average obtained, 15.64 per cent, checked very closely with the theoretical, 15.65, but the variation was 0.61 per cent. It will be noted that although the prepared product stood overnight at room temperature, the results give no indication of any distinct settling of moisture.

That other analysts are encountering difficulty with the official method is well brought out by D. B. Bisbee<sup>1</sup> in a study of the records of analyses of some 1500 samples of butter made by various analysts. He records duplicate determinations of fat varying by 0.26, 0.56, and 0.42 per cent, and of moisture, 0.30, 0.21, and 0.25 per cent. He cites composite samples, in which the fat varied from the average of the fats determined on the individual subdivisions from which the composite was prepared by 0.50, 0.48, and 0.20 per cent. The moisture on these same

<sup>1</sup> Unpublished report.

composite samples varied from the average of the moisture of the individual units by 0.18, 0.47, and 0.25 per cent. The solids not fat in these composites varied from the average of the solids not fat of the individual units from which the composites were prepared by 0.67, 0.02, and 0.43 per cent. Bisbee states that there were cases in which duplicate determinations varied more than 1 per cent.

The work described in this paper clearly demonstrates, in the opinion of the writers, that for a product as complex physically as butter it is impossible to get check results by a method in which there is so much room for slight differences in technique. Since physical conditions, particularly the temperature, affect the homogeneity of the product so greatly, a satisfactory method should make provision for every possible variation.

TABLE 6.  
*Comparison of moisture results obtained by using two methods.*

SAMPLE NUMBER	METHOD OF PREPARATION		AVERAGE DIFFERENCE— OFFICIAL IN EXCESS OF COMMERCIAL
	OFFICIAL <i>per cent</i>	COMMERCIAL (26°C.) <i>per cent</i>	
1	15.60	15.06	0.490
	15.54	15.10	
2	14.59	14.37	0.210
	14.75	14.55	
3	17.58	17.43	0.090
	17.49	17.46	
4	11.31	11.02	0.470
	11.61	10.92	
5	20.53	19.95	0.490
	20.46	20.06	
6	13.29	13.07	0.395
	13.53	12.96	
7	14.70	14.51	0.145
	14.62	14.52	
8	15.07	14.69	0.405
	15.12	14.69	
9	14.37	14.33	0.110
	14.37	14.19	
10	15.18	14.52	0.570
	15.04	14.56	
Average	15.24	14.90	0.337
Among duplicates			
Maximum difference	0.30	0.18	
Average difference	0.12	0.07	

#### COMMERCIAL METHOD.

Dairy chemists and buttermakers, who seldom use the official method for the preparation of a sample for the moisture determination, in the

belief that it is unreliable and impracticable, have long used what may be designated the "commercial" method. In this method a sample of butter is beaten to a creamy consistency by means of a spatula, knife, spoon, or other suitable instrument.

In May, 1924, L. C. Mitchell made a report (unpublished) of a study of the official and commercial methods for the determination of moisture, a part of which is here incorporated.

Table 6 shows a comparison of the two methods. Ten samples of butter were analyzed. For the commercial method, the butter was beaten to a creamy consistency at 26°C. by means of a spatula and a portion was removed for analysis; the butter was again beaten, and a duplicate portion was taken. The samples were then prepared by melting and shaking under a stream of cold water until they solidified. Duplicate portions were then taken from opposite sides of the containers for analysis. The results are given in Table 6.

Although the commercial method gave closer check results, the official method gave consistently higher moisture values, the excess being from 0.11 to 0.57 per cent, averaging 0.34.

It was observed that in the commercial method the butter failed to "wet" the surface of the container, indicating the probability that some of the moisture adhering to the sides of the jar was not incorporated with the sample. Attempt was made to obviate this difficulty by the use of a higher temperature.

TABLE 7.  
*Comparison of moisture results obtained by two methods.*

SAMPLE NUMBER	METHOD OF PREPARATION		AVERAGE DIFFERENCE	
	OFFICIAL	COMMERCIAL (29°-31°C.)	OFFICIAL IN EXCESS OF COMMERCIAL	COMMERCIAL IN EXCESS OF OFFICIAL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	17.81	17.93		
	17.74	17.86		0.120
2	18.84	18.69		
	18.87	18.67	0.175	
3	18.44	18.16		
	18.50	18.20	0.290	
4	24.85	24.77		
	24.78	24.83	0.015	
5	22.34	22.17		
	22.22	22.10	0.145	
6	25.10	25.35		
	25.20	25.40		0.225
7	26.24	26.37		
	26.38	26.22	0.015	
8	26.54	26.51		
	26.65	26.59	0.045	
Average	22.53	22.49	0.043	

Eight samples were prepared in the manner described above, except that in the commercial method the samples were beaten at 29°-31°C., the butter barely wetting the sides of the container. The moisture results are given in Table 7.

The official method gave moisture values averaging 0.04 per cent higher than those obtained by the commercial method, but two of the eight samples showed higher values by the latter method. A slightly higher temperature would probably have caused complete wetting, with incorporation of all the moisture into the butter.

To test the homogeneity of a sample prepared by the commercial method, 17 moisture determinations were run on one sample prepared as follows:

A pound of butter was warmed until one-sixth had melted, then beaten to a creamy consistency by means of a spatula, the final temperature of the mixture being 33°C. The butter completely wetted the sides of the container. Six, six, and five samples, respectively, were taken from the upper, middle, and lower portions of the product and analyzed. The results given in Table 8, though showing a maximum variation of 0.20 per cent for moisture, are distinctly better than those obtained by the official method on a similar number of samples.

TABLE 8.  
*Results showing homogeneity of sample prepared by  
"commercial" method at 33°C.*

LAYER	MOISTURE	SOLIDS NOT FAT	FAT (BY DIFFERENCE)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Upper	15.79		
	15.78		
	15.65		
	15.63		
	15.69	4.30	80.01
	15.70	4.35	79.95
Variation	0.16	0.05	0.06
Middle	15.75		
	15.75		
	15.69		
	15.75		
	15.80	4.34	79.86
	15.70	4.34	79.96
Variation	0.11	0.00	0.10
Lower	15.78		
	15.64		
	15.60		
	15.68		
	15.78	4.40	79.82
	0.18		
Maximum	15.80	4.40	80.01
Minimum	15.60	4.30	79.82
Average	15.71	4.35	79.92
Total variation	0.20	0.10	0.19

It is apparent that the commercial method gives greater uniformity of results than does the official, and, provided the butter is beaten up at 31°-34°C., yields as high moisture results.

It is a fairly well-known fact that dairy chemists and especially buttermakers generally obtain lower moisture results than those found by food control laboratories. The probable explanation is found in the observation of the writers, that in many instances the buttermakers do not beat up the butter at a temperature sufficiently high to cause it to wet the sides of the container.

#### STIRRER METHOD.

Since the results obtained in both the official and commercial methods depend somewhat on the personal judgment and muscular energy of the analyst, a more ideal method will involve less of the human element and be less time-consuming and laborious.

The method worked out depends on the use of a malted milk mixer or stirrer<sup>1</sup>, such as is used by soda fountains, and is here termed the "stirrer" method.

TABLE 9.  
*Results obtained by "stirrer" method.*

SAMPLE NUMBER	MOISTURE <i>per cent</i>	SOLIDS NOT FAT <i>per cent</i>	FAT (BY DIFFERENCE) <i>per cent</i>
1	13.71	3.58	82.73
	13.69		
2	13.93	3.56	82.51
	13.94	3.59	82.47
3	14.07	3.55	82.38
	14.01	3.65	82.34
4	13.98	3.58	82.44
	13.98	3.63	82.39
5	14.41	3.61	81.98
	14.41	3.60	81.99
6	14.02	3.54	82.44
	14.09	3.47	82.44
7	14.31	3.75	81.94
	14.24	3.78	81.98
8	14.21	3.79	82.00
	14.12	3.76	82.12
9	14.40	3.92	81.68
	14.30	3.86	81.84
Variation	0.10	0.10	0.16

Some of the results obtained by the use of this method are given in the following experiments:

<sup>1</sup> Hamilton Beach Mfg. Co., Racine, Wisc., Type No. 2.



Nine samples of butter consisting of one pound prints placed in quart Mason jars were warmed until approximately one-half of each sample was melted, and then stirred for two minutes by means of the malted milk stirrer. The butter assumed a creamy consistency, and the volume increased about 20-30 per cent. The final temperature of the stirred samples was 32°-34°C., and the product thoroughly wetted the inner surface of the jar. After allowing to stand at room temperature for 3-4 hours, duplicate samples were taken from the surface on opposite sides of the jar. The figures, given in Table 9, show the uniformity of results obtained.

TABLE 10.  
*Results showing homogeneity of sample of "sweet" butter prepared by "stirrer" method.*

LAYER	MOISTURE	SOLIDS NOT FAT	FAT (BY DIFFERENCE)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Upper	10.33	0.51	89.16
	10.33	0.51	89.16
	10.34	0.51	89.15
	10.30	0.53	89.17
	10.29	0.52	89.19
Middle	10.37	0.54	89.09
	10.31	0.54	89.15
	10.32	0.52	89.16
	10.30	0.52	89.18
	10.35	0.52	89.13
Lower	10.41	0.54	89.05
	10.41	0.53	89.06
	10.43	0.51	89.06
	10.32	0.53	89.15
Variation	0.14	0.03	0.14

In another experiment, a pound of "sweet" butter was prepared in the same manner, the final temperature of the mixture being 33°C. The jar was kept in a refrigerator for one hour. Five samples were taken from the upper surface, five from the middle, and four from the bottom of the jar. The homogeneity of the prepared sample is shown in the results given in Table 10.

TABLE 11.  
*Moisture results showing homogeneity of sample of butter of known composition prepared by "stirrer" method.*

	UPPER LAYER	MIDDLE LAYER	LOWER LAYER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	15.01	14.99	14.98
	14.95	14.91	14.92
	14.95	15.02	15.04
	14.90	14.96	14.91
	15.07	14.96	15.04
Variation	0.17	0.11	0.13
Maximum		15.07	
Minimum		14.90	
Average		14.97	
Total variation		0.17	
Theoretical		14.96	

A sample of "synthetic" butter containing 14.96 per cent water was warmed to 33°C., then stirred. The consistency of the resultant product was such that it was necessary to cool and stir alternately until the butter reached a temperature of 19°C., before it had the consistency of natural butter at 33°C. The butter completely wetted the surface of the jar. Five samples were taken from each of three layers, as in previous experiments. The average of fifteen determinations for moisture is 14.97 per cent, as against the 14.96 per cent put into the butter. The maximum variation is 0.17 per cent.

#### SUMMARY AND CONCLUSION.

The official method of preparing butter for analysis discussed in this paper, is indefinite, inaccurate, laborious, and time-consuming.

The "commercial" method, while yielding more uniform results than the official method under the proper conditions, is laborious.

The "stirrer" method gives more uniform, accurate, and reliable results than either of the other two methods, and is considerably quicker and less laborious.

The details of the proposed method are as follows:

Warm the sample, 250-500 grams, in a closed vessel until about half of it is melted. Stir with a malted milk mixer for 2-3 minutes, with up-and-down movement of the stirring device. The final temperature must be 31°-34°C., at which temperature the butter will completely wet the sides of the container. If the temperature is below 31°C., continue stirring until this temperature is reached. A temperature above 34°C. will indicate that the sample has been warmed too much. In this case, cool the sample until solid and repeat the warming and mixing.

#### DETERMINATION OF THE TOTAL SOLIDS OF BREAD.

By RAYMOND HERTWIG and L. H. BAILEY (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

The U. S. Department of Agriculture definitions and standards<sup>1</sup> for wheat bread, milk bread, and rye bread stipulate that they contain, "one hour or more after baking, not more than thirty-eight per cent (38%) of moisture, as determined upon the entire loaf or other unit". The present tentative method for the determination of moisture in baked cereal products<sup>2</sup>, directs that the sample be reduced to pass a 60-mesh sieve. Since material moisture losses would occur during such reduction of the bread, it is evident that the method does not determine the true moisture content of the entire original loaf. This investigation was undertaken, therefore, to develop a method for the determination of the total solids of an entire loaf or other unit of bread that would be accurate, readily

<sup>1</sup> Food Inspection Decision 188.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 230.

adaptable to ordinary laboratory practice, and practical for general use.

To ascertain the total solids of an entire loaf or other unit of bread two more or less independent procedures are necessary—the determination of the loss in weight by evaporation during the preparation of the sample for analysis and the determination of the loss in weight in the prepared sample by oven-drying or other appropriate method. The total solids of the entire unit of bread are calculated from the original weight of the bread, the weight after air-drying in preparation for grinding the sample, and the percentage of total solids of the prepared ground sample.

The most complete and detailed method found in chemical literature for the determination of the total solids of bread loaves—that of the German Imperial Bureau of Health<sup>1</sup>—requires that the loaf be weighed immediately upon receipt and again when the analysis is started. The loaf is cut into four symmetrical parts, one of which is used for the analysis. This quarter is accurately weighed and then cut into slices 2–3 mm. thick, which are spread out on suitable trays and either dried 8 hours in an oven or overnight in a warm room and then 2 hours in an oven. After the partially dried slices have attained equilibrium with the room atmosphere—in order to prevent any weight changes during grinding—they are carefully weighed and then ground to a fine powder. The moisture is determined in 10 grams of the mixed ground material by drying in an oven at 100°C. to constant weight. The moisture content of the entire loaf of bread at the time of its receipt is calculated from the losses in weight of the whole loaf before beginning the analysis, of the quarter loaf during the preparation of the ground sample, and of the ground sample during the final oven drying, all losses being calculated to the same basis of the original bread material.

Some modifications of the preceding method seemed desirable to adapt it to the requirements of the bread standards of the Department of Agriculture and to routine laboratory practice.

The loaf or other unit of bread should not be weighed sooner than one hour after baking in order to conform to the requirements of the official standards. Otherwise the sample should be accurately weighed immediately upon receipt, or, when accurate weighing at this time is impossible, it should be sealed in an air-tight container and weighed as soon thereafter as possible. Precautions must be taken to prevent material losses to the sample after weighing, as such losses would be calculated subsequently as moisture.

The use of a quarter loaf instead of the entire loaf for determining the total solids is not advisable because the crust is not homogeneously distributed, especially about multiple loaves and loaves with an oven break; irregular distribution of the moisture in a loaf naturally accompanies

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<sup>1</sup> *Arb. kais. Gesundh.*, 1915, 48: 505.

irregular distribution of the crust. Consequently the total solids of a loaf of bread should be determined from a sample representative of the entire loaf.

For the purposes of the method outlined in this paper, it is essential that the total solids of the air-dried sliced bread be the same as those of the air-dried ground bread, as losses in weight that occur during the grinding are not incorporated in the final results. In this connection it was found that sliced bread spread out on a paper in a warm room until it is crisp and brittle does not lose any consequential weight when ground to pass a 20-mesh sieve. Weight losses occurring during the grinding of eight commercial loaves, representing six different types of breads that had been sliced and exposed to the air for about 16 hours, or until crisp and brittle, are given in Table 1.

TABLE 1.  
*Weight losses during air-drying and grinding of breads.*

TYPE OF BREAD	WEIGHT OF LOAF	WEIGHT AFTER AIR- DRYING AND BEFORE GRINDING	WEIGHT AFTER GRINDING TO PASS A 40-MESH SIEVE	WEIGHT AFTER GRINDING TO PASS A 20-MESH SIEVE	LOSS IN WEIGHT DURING GRINDING	WEIGHT LOSSES DURING GRINDING AS PER- CENTAGES OF WEIGHTS OF ORIGINAL LOAVES
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	
White. . . . .	432.0	316.0	314.0	. . .	2.0	0.46
Vienna. . . . .	485.0	370.0	370.0	. . .	0.0	0.00
Three-fourths rye . . .	703.0	493.0	488.0	. . .	5.0	0.71
Bran . . . . .	467.0	325.0	321.0	....	4.0	0.85
Graham . . . . .	455.0	320.0	319.0	....	1.0	0.22
White. . . . .	434.0	314.5	....	314.0	0.5	0.11
White . . . . .	424.0	303.5	....	303.0	0.5	0.12
Pumpernickel. . . . .	694.0	482.5	....	481.5	1.0	0.14

The results in Table 1 show that the differences between the total solids content of the partially dried sliced breads, prepared in the manner indicated, and that of the respective samples ground to pass a 20-mesh sieve are so slight that they may be disregarded in the final calculation of the total solids of the original bread loaf. This fact permits the convenient determination of the total solids of entire bread loaves by estimating only the loss in weight during preliminary air-drying of the sliced loaf and the total solids of the ground air-dried slices. On the other hand, repeated grindings necessary to pass the sample through a 40-mesh sieve may cause consequential losses in some instances.

The method of the German Bureau of Health determines the total solids of the prepared ground bread by drying a 10 gram portion to constant weight in an oven at 100°C. Drying at 100°–105°C. in an air oven to constant weight is the usual procedure for this determination in bread as found in numerous texts on the analysis of foods. Since the Association of Official Agricultural Chemists is considering the adoption of a proposed umpire vacuum method<sup>1</sup> that stipulates drying at 98°–100°C. in a partial vacuum having a pressure of not more than 25 mm. of mercury, as the standard method for determining total solids in all cereal products, these specifications have been used as a standard in this investigation.

TABLE 2.  
*Total solids in ground air-dried bread.*

TYPE OF BREAD	PROPOSED UMPIRE VACUUM METHOD 98°–100°C., 15–20 MM. HG PRESSURE		AIR-OVEN DRYING RESULTS—TEMPERATURE 114°–116°C.					
			2 hours drying		3 hours drying		2 hours drying	4 hours drying
			Sample ground to pass 40-mesh sieve		Sample ground to pass 20-mesh sieve		Sample ground to pass 20-mesh sieve	Sample ground to pass 20-mesh sieve
			(A)*	(B)*	(A)*	(B)*		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
White . . . . .	89.09 89.10	. . . .	89.15 89.17	89.11 89.12	. . .	. . .	. . .	. . .
Vienna . . . . .	91.16 91.20	. . .	91.19 91.22	91.27 91.29	. . . .	. . . .	. . .	. . . .
Three-fourths rye	89.97 89.97	. . . .	89.99 89.99	90.05 90.07	. . . .	. . . .	. . . .	. . . .
Bran . . . . .	90.19 90.19	. . . .	90.36 90.39	90.21 90.24	. . . .	. . . .	. . . .	. . . .
Graham . . . . .	90.37 90.40	. . . .	90.32 90.37	90.43 90.46	. . . .	. . . .	. . . .	. . . .
White . . . . .	90.65 90.68	90.53 90.55	. . .	. . . .	90.63 90.65	90.61 90.62	90.68 90.88	90.46 90.51
White . . . . .	90.20 90.21	90.07 90.07	. . . .	. . . .	90.19 90.20	90.13 90.17	90.19 90.21	89.99 90.06
Pumpernickel . . .	90.67 90 68	90.47 90.47	. . . .	. . . .	90.40 90.44	90.28 90.31	90.42 90.46	90 20 90.21

\* (A) (B) Dried on different days

NOTE.—Approximately 2-gram sample portions were used in all determinations.

Since this proposed umpire vacuum oven method is not economical for general use, study was made of a possible substitute routine method that would yield approximately the same results. Inasmuch as such a

<sup>1</sup> To be published in the Nov. 15, 1925 issue of *This Journal*.

method, involving air-oven drying at 130°C. for one hour, has been proposed for the moisture determination of flour<sup>1</sup>, study was made of its applicability to ground bread. However, it was found that ground bread dried at 130°C. for one hour, or even for one-half hour, undergoes a marked darkening in color, while bread dried at 115°C. for 3 or 4 hours undergoes only slight darkening. Consequently, the applicability of a temperature of 115°C. was further studied.

Two-gram portions of ground bread were dried under the conditions listed in Table 2. The results obtained by air-oven drying at approximately 115°C. are tabulated along with those obtained by the proposed umpire vacuum oven method.

The results of the experiments recorded in Table 2 show that different types of bread ground to pass a 20-mesh sieve and dried in an air oven at approximately 115°C. for from 2-3 hours give results closely approximating those obtained by the proposed umpire vacuum oven method. Drying for 4 hours yields only slightly different results. Slight losses of moisture occur when the ground air-dried bread passing a 20-mesh sieve is reground to pass a 40-mesh sieve, indicating that such finer grinding is to be avoided.

The results in Table 3, calculated from the data of Tables 1 and 2, give the total solids of the eight loaves of bread analyzed in this investigation.

TABLE 3.  
*Total solids of commercial breads.*

TYPE OF BREAD	PROPOSED UMPIRE VACUUM METHOD	AIR-OVEN DRYING AT 115°C.
	<i>per cent</i>	<i>per cent</i>
White . . . . .	63.38	63.34
White . . . . .	65.50	65.58
White . . . . .	64.36	64.46
Vienna . . . . .	69.63	69.57
Three-fourths rye . . . . .	62.43	62.47
Bran . . . . .	61.99	62.12
Graham . . . . .	63.37	63.34
Pumpernickel . . . . .	62.76	62.71

The following methods are proposed for the determination of the total solids of an entire loaf or other unit of bread:

#### DETERMINATION OF THE TOTAL SOLIDS IN BREAD.

("Loaf" refers to a loaf or other unit of bread.)

Accurately weigh the loaf of bread immediately upon receipt (A). Use scales sensitive to at least 0.3 gram. (When determining whether bread is in conformity with the Department of Agriculture standards do not weigh the loaf sooner than one hour after removal from the oven.) Should accurate weighing be impossible at this time, seal the sample

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 301.

in an air-tight container and accurately weigh as soon thereafter as is practicable (A). Preserve the sample in such a manner that no loss of bread solids can occur, whereby the loss would be calculated as moisture. Cut the bread into slices 2–3 mm. thick. Spread the slices out on paper, allow to dry in a warm room (approximately 15–20 hours), and when apparently dry, break into fragments. If the bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with the moisture of the air—in order that no moisture changes may occur during grinding. Quantitatively transfer the air-dried bread to the scale pan and accurately weigh (B). Grind the sample to pass a 20-mesh sieve, mix well, and keep in an air-tight container. Determine the total solids in the ground sample by one of the following methods:

*Proposed Umpire Vacuum Method.*

APPARATUS.

(a) *Aluminum dish*.—Diameter about 55 mm., height about 15 mm., provided with an inverted slip-in cover fitting tightly on the inside.

(b) *Air-tight desiccator*.—Should contain reigned quick lime or calcium carbide.

(c) *Vacuum oven*.—Should be connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm. or less of mercury, and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. A gas drying bottle containing concentrated sulfuric acid is connected with the oven for admitting dry air when the vacuum is released.

(d) *A mercury manometer*.—Used to indicate the pressure of the partial vacuum.

DETERMINATION.

Weigh accurately about 2 grams of the well mixed, ground sample in a covered dish that has been dried previously at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature. Loosen the cover (do not remove) and heat at 98°–100°C. to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm. or less of mercury. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temperature is attained. From the weight of the residue calculate the percentage of total solids of the ground sample portion (C).

*Routine Method.*

APPARATUS.

(a) *Metal dish and desiccator*.—Same as in the vacuum oven method.

(b) *Oven*.—Capable of maintaining a temperature of 112°–117°C. and provided with an opening for ventilation.

(c) *Thermometer*.—To be placed with its bulb near the samples to indicate the oven temperature.

DETERMINATION.

Weigh accurately approximately 2 grams of the well mixed, ground sample in a covered dish that has been dried previously at 112°–117°C., cooled in the desiccator, and weighed soon after attaining room temperature. Uncover the sample and dry in the oven at 112°–117°C. for approximately 3 hours. Cover the dish, transfer to the desiccator, and weigh soon after room temperature is attained. From the weight of the residue, calculate the percentage of total solids of the ground sample portion (C).

*Calculation of Total Solids in Bread Loaf.*

Calculate the total solids of the original entire bread loaf as follows:

Let  $A$  = Weight of the loaf at time of receipt;

$B$  = Weight of the air-dried sliced loaf of bread;

and  $C$  = Percentage of total solids in the prepared ground sample.

Then the percentage of total solids of the original entire loaf =

$$\frac{\frac{B \times C}{100} \times 100}{A} \text{ or } \frac{B \times C}{A}.$$





## SECOND DAY.

### TUESDAY—MORNING SESSION.

#### REPORT ON CHEMICAL REAGENTS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Referee*.

It is not difficult as a rule to decide whether or not a chemical is fit for use as a reagent; usually any impurity is easily detected. It is the samples that are *almost* good enough that give trouble in making decisions as to their acceptability.

The Bureau of Chemistry has received 118 reagents for test since November 1, 1923. Seven of these have been rejected for the following reasons:

*Ammonium thiocyanate*.—High in non-volatile matter.

*Lead carbonate*.—Contained 1.2 per cent water-soluble matter.

*Silver nitrate*.—Was not clear white. Twenty-nine hundredths of a per cent of it was not precipitable by hydrochloric acid.

*Ammonium chloride*.—The non-volatile residue from this salt was 0.03 per cent, and it contained 0.06 per cent of sulfate.

*Acetic acid, 99.9 per cent*.—Had the correct acid strength but was contaminated by sediment in the bottles.

*Benzene*.—Carbon disulfide was present in excess.

*Magnesium oxide*.—The water-soluble content of this material amounted to 16 per cent of its weight. It was free from sulfur but contained 2.36 per cent of chlorine.

The Federal Specifications Board has already undertaken through one of its subcommittees to define the nature and extent of permissible impurities in chemicals. The undertaking as a whole is so extensive that progress will necessarily be slow. Special attention is being paid at present to photographic chemicals.

Steps have also been taken toward the closer cooperation of bureaus in the Department of Agriculture for the purpose of securing greater uniformity in the quality of chemicals supplied to the various laboratories.

#### RECOMMENDATION.

It is recommended that the observations on reagent chemicals be continued and that collaborative work be instituted if possible.

## REPORT ON EGGS AND EGG PRODUCTS.

By RAYMOND HERTWIG (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

In accordance with the recommendations adopted at the 1923 meeting of the association<sup>1</sup>, methods for the analysis of liquid, frozen, and dried eggs have been studied.

Since the association has no methods for the examination of eggs, the purpose of the work undertaken was to collate any available fundamental methods, subject them to the study of the referee, the associate referees, and the collaborators, and prepare for adoption those found commendable. The studies contemplated were divided among the four following associate referees, all members of the Bureau of Chemistry: M. L. Hitchcock, Chicago Station; J. C. Palmer, San Francisco Station; W. E. Kirby, New York Station; and H. I. Macomber, New York Station.

Methods for moisture, ash, fat (acid hydrolysis method), lipoids, lipid phosphoric acid ( $P_2O_5$ ), acidity of fat, acid-soluble phosphoric acid, organic and ammoniacal nitrogen, water-soluble protein-nitrogen precipitable by 40 per cent alcohol, preservatives, and zinc were assigned for study.

Eggs are marketed as shell eggs, as shelled whole egg, as separated commercial egg white and commercial egg yolk, and as admixtures of the two latter forms. Egg material comes into commerce as frozen egg and dried egg in tin containers, barrels, and tin-lined boxes. The dried egg may be in flake or powdered form. Each of the different physical conditions of eggs requires special consideration for sampling and chemical analysis.

### DISCUSSION OF THE METHODS STUDIED AND OF THE REPORTS AND RECOMMENDATIONS OF THE ASSOCIATE REFEREES.

#### *Taking and Preparation of Sample.*

Methods for the taking and preparation of samples of powdered dried egg and liquid or frozen egg were studied by Associate Referees Hitchcock and Palmer. Resulting from their comments and recommendations, methods are presented for sampling these products in the final recommendations of this report.

#### *Determination of Total Solids.*

Palmer gave careful study to the determination of total solids in powdered dried eggs at the temperature of 98°C. and at the respective pressures of 6 inches and 2½ inches, and also at the temperature of 55°C. and at the pressure of 2½ inches. Considerably higher results for total

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 273.

solids were obtained at 55°C. and at the pressure of 2½ inches than at 98°C. and at either of the pressures of 6 inches or 2½ inches. Slightly lower results as total solids were obtained at 2½ inches than at 6 inches pressure. At the temperatures and pressures studied, practically the entire loss in weight occurred within 3½ hours. Very minor progressive losses were found to take place between 3½ and 5½ hours. Constant weight was assumed after about 5 hours.

Hitchcock carefully studied the total solids determination for liquid eggs as just described for dried eggs. He found the temperature of boiling water gave markedly lower results as total solids than a temperature of 55°C. Practically the entire loss in weight occurred within 3½ hours, but very minor progressive losses continued up to 5 hours.

Deductions drawn from the studies of the two associate referees are that the temperature and pressure conditions must be definitely stipulated in the method for determining total solids in eggs to obtain strictly duplicable results. The loss in weight is apparently a function of the temperature and pressure conditions.

It is believed necessary for the association to have two methods for the total solids determination in eggs: First, a standard umpire method giving results as near to the absolute actual solids as are practically obtainable. Such a method may not at the same time be readily applicable in ordinary practice because of its lack of economy of time and outlay of apparatus, but it serves as a standard by which to judge the merits of the more practical and possibly slightly less accurate methods. Second, a rapid, simple, practical method for routine work involving ordinary chemical analyses and giving results very close to those of the standard umpire method in a short time and with little outlay in apparatus.

Attention is here directed to the report of this referee acting as Referee on Cereal Foods, in this number of *The Journal*, and to that part pertaining to the determination of moisture in flour. For the reasons there given, in addition to the observations made by the associate referees, it is believed that a standard umpire method similar to the one recommended for adoption for flour should be adopted as official for determining total solids in eggs. A rapid method for this determination similar to the rapid routine method recommended for moisture in flour should be studied during the coming year. In this connection the referee and L. H. Bailey of the Bureau of Chemistry obtained 74.22 and 74.18 per cent total solids in a sample of liquid eggs by the standard umpire method just mentioned for moisture in flour, and 74.12 and 74.18 per cent by the rapid routine method for moisture in flour, which results indicate the possibility of employing such a rapid method for eggs.

*Determination of Organic and Ammoniacal Nitrogen.*

Palmer studied this determination on dried egg by the three official methods. He reports the most favorable results in the shortest time by the Kjeldahl-Gunning-Arnold method. This method is recommended also for the same reasons by the Nitrogen Laboratory of the Bureau of Chemistry. Hitchcock obtained excellent collaborative results by this method on liquid eggs. The method proposed in the recommendations of this report includes certain stipulations suggested by Palmer and Hitchcock.

*Determination of Fat by the Acid Hydrolysis Method.*

It has been customary to determine the crude fat or ether extract of eggs by extracting a dried portion with anhydrous ether. As the referee<sup>1</sup> has shown that this method does not extract all the ether-soluble substances, he proposes a method for extracting the fat after the hydrolysis of the sample with acid. Considerably more fat is extracted in this manner.

In his study of this method with powdered dried egg Palmer found that the temperature range, 75°–80°C., for the acid hydrolysis yielded slightly higher results compared with those obtained at other temperatures. His investigation of the method shows it to be satisfactory for powdered dried egg. Experimental error is high in the determination of fat in dried eggs, as the fat content constitutes such a large proportion of the sample; therefore, slight variations in the results may at times be expected. Palmer did not have opportunity to obtain collaborative results by this method on dried eggs, but judging from his thorough study and the satisfactory results reported it is thought that the method has been sufficiently tried out to recommend it at this time for adoption as a tentative method for powdered dried egg.

Hitchcock studied the method in connection with liquid eggs, obtained excellent collaborative results, and recommended it for adoption as a tentative method for liquid eggs.

*Determination of Lipoids and Lipoid Phosphoric Acid ( $P_2O_5$ ).*

This determination was devised by the referee<sup>2</sup> to extract completely all fat-like substances from egg, flours, and alimentary pastes in as unchanged a condition as any known methods will permit. In most instances the method determines more of such substances than any other known method. Neutral extractives are used to avoid any possible decomposing actions of acids and of alkalies. The method extracts true fats, fatty acids, unsaponifiable matter, sterols, lecithin and similar substances, coloring matter, and waxes.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 508.

<sup>2</sup> *Ibid.*, 7: 91.

Palmer studied the method in connection with powdered dried eggs and obtained excellent results. To obtain complete extraction, he recommends the use of a 2 gram sample, the extraction of the excess fat, and then its reduction to a fine powder in admixture with precipitated calcium carbonate in a mortar. Although Palmer had no opportunity to obtain collaborative results, in this case it is thought to be unnecessary for its recommendation for adoption as a tentative method for powdered dried eggs in view of the careful study and the excellent results reported by him.

Hitchcock also obtained excellent collaborative results for the method as adapted to liquid eggs and recommends its adoption as a tentative method.

#### *Determination of the Acidity of Fat.*

This determination is useful in connection with establishing the edible quality of the egg material. The acidity is reported in terms of cc. of 0.05 *N* sodium ethylate per 1 gram of fat or extract. For the intended purposes of the determination it is desirable that the acidity of the fat be made on a complete extraction of all fat-like substances of the sample and that the results reported be based on such a complete extract.

Methods of different efficiency for extracting the fat-like substances in eggs can not be expected without experimental demonstration to yield similar results for this acidity determination. It has been customary to determine acidity of fat in eggs by titrating the extract from the direct extraction of the dried sample with anhydrous ether. This extraction method is acceptable from the standpoint of operation but the extraction is incomplete, and to that extent the results may not depict the true acidity, although no data are at hand to substantiate this inference. A method that will extract more fat-like substances than this direct method may be expected to give different results. The method for lipoids considered in this report yields the most complete extraction of fat-like substances from eggs of any known method, and in this respect it should be commendable for the determination of acidity of fat. It is thought that some modification of the lipid method could be made to incorporate the determination of the acidity of the lipoids, which would allow correct expression of the results on the basis of one gram of all the fat-like substances.

Associate Referee Macomber in his study of the two extraction methods in connection with the determination for acidity of fat found that the lipid extract darkens during the drying at 100°C. to such an extent as to interfere with the titration color changes. He considers the lipid method as taking more of the analyst's time than the direct ether extraction method and also that the incomplete extraction of fat is inconsequential for the determination of the acidity of the fat. Macomber sub-

mitted the acidity of fat determination by the direct anhydrous ether extraction method to collaborative study. The results for dried eggs are satisfactory, but those for liquid eggs differ very considerably in some instances. He recommends that the method be adopted tentatively and that it be further studied.

Effort should be made to keep the methods of egg analysis as concordant as is consistent with practicability and accuracy, and no methods or parts of methods should be incompatible with other methods or parts of methods. The determination of acidity of fat as given in the method recommended for adoption by the associate referee incorporates total solids determination by drying the sample in vacuo at 55°C. and the extraction of the fat in a manner that does not obtain all fat-like substances. Neither of these latter methods is in harmony with other methods that at this time are being recommended for adoption as tentative and that are believed to be more appropriate for the determination of these respective substances.

*Determination of Water-Soluble Protein-Nitrogen Precipitable  
by 40 Per Cent Alcohol.*

This method was first devised by the writer<sup>1</sup> to distinguish between egg alimentary pastes made with commercial egg yolk from those made with whole egg and to aid in estimating the egg solids content. The method determines essentially the albumin of such products as noodles, eggs, flours, etc. It differentiates between whole egg material and material from which egg white has been separated to a greater or less degree. The method promises to be serviceable in egg and egg product analyses where information regarding the albumin content is desired.

Hitchcock reported quite satisfactory collaborative results by the method on liquid eggs. Palmer studied the method as applied to powdered dried eggs and found some difficulty in filtering the precipitated albumin. He proposes to modify the method by determining the nitrogen in the albumin indirectly by difference so as to eliminate the filtering of the precipitate. He also noted that the nitrogen of the water-soluble extract before the addition of the alcohol to throw out the albumin was not so constant as is desirable, which fact he attributed to the difficulty with which albumin diffuses into distilled water. The extraction was found to be more satisfactory with a 1.2 per cent salt solution into which albumin diffuses more readily than into pure water. The modifications proposed by Palmer appear worthy of further study. In this connection, it is recommended that the separation and washing of the precipitated albumin from the mother liquid by centrifugalizing and decantation be considered and that the nitrogen in the precipitate be determined directly

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 84.

to avoid the additional errors of an indirect method. Separation of the precipitate from the mother liquid by centrifugalizing may be brought about by the addition of finely divided asbestos fiber to cause the flocculent precipitate to pack well. The supernatant liquid can then be decanted off and the residue washed in the same manner.

#### *Determination of Acid-Soluble Phosphoric Acid.*

The determination of acid-soluble phosphoric acid was proposed by Pine<sup>1</sup>, as a means to differentiate chemically between edible eggs and eggs in various stages of decomposition. He found that a dilute solution of hydrochloric acid and picric acid extracts considerably different quantities of phosphoric acid from edible and inedible eggs. Macomber was able to simplify the method somewhat by shortening the time of extraction and by separation of the insoluble egg material from the extract by centrifugalization and decantation instead of by filtration. He also eliminated the first precipitation of the phosphoric acid as ammonium phosphomolybdate in the determination of the phosphoric acid and found that a single direct precipitation as magnesium ammonium phosphate did not affect the results.

The modified method was submitted to collaborative study. The results obtained are not so satisfactory as they should be for the application of the method for its intended purposes. The associate referee believes the variable results due to insufficient mixing of the acid and sample and recommends further study of the method.

#### *Determination of Zinc.*

Associate Referee W. E. Kirby has undertaken the study of methods for determining zinc in eggs. This determination as zinc sulfide has not been altogether satisfactory because of difficulty in filtering, but Kirby proposes the determination as a zinc-mercury-thiocyanate. He has studied the procedure of the method, but as yet has not developed it to that point of completion where it can be submitted for collaborative study.

#### RECOMMENDATIONS<sup>2</sup>.

It is recommended—

(1) That the following methods be adopted as tentative for the taking and preparation of samples of liquid, frozen, and powdered dried egg:

##### *Taking and Preparation of Sample.*

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material as the conditions encountered may differ

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 57.

<sup>2</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 273.



widely. Experienced judgment must be called upon in each instance. Generally speaking, where large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid Eggs*: Secure representative container or containers. Mix the contents of a container thoroughly and draw about a 300 gram sample for analysis. A long handled dipper or ladle serves well to withdraw the sample. Keep the sample hermetically sealed in a jar in a cool place. Report odor and appearance and examine for foreign material.

(b) *Frozen Eggs*: Secure representative container or containers. Examine contents as to odor and appearance and for foreign material. Condition of contents can be determined best by boring a hole to the center of a container with an auger and noting the odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings made on the contents of each container. Borings should be taken midway between the center and circumference of the top of the can from at least three widely separated parts and should extend to as near the bottom of the can as possible. Collect about 300 grams of the sample. Keep hermetically sealed in a jar in a cool place and in a frozen state if possible. When ready for analysis, warm the samples in a bath held below 50°C. and mix well.

(c) *Powdered Dried Eggs*: Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove the top layer to a depth of about six inches with a flour scoop or other convenient instrument. Draw small quantities of sample totaling about 300–500 grams from accessible parts of the container and place in a hermetically sealed jar. Report odor and appearance and examine for foreign material. Prepare the sample for analysis by mixing three times through a domestic flour sifter to assure complete breaking up of lumps. Keep in a hermetically sealed jar in a cool place.

(2) That study be made during the coming year of the preparation of samples of flake dried eggs for analysis by the methods of this report.

(3) That the following method for the determination of total solids in liquid eggs and powdered dried eggs be adopted as official: (First action.)

*Total Solids—Umpire Vacuum Method.*

APPARATUS.

(a) *Metal dish*.—Diameter about 55 mm., height about 15 mm., provided with an inverted slip-in cover fitting tightly on the inside.

(b) *Air-tight desiccator*.—Should contain reignited quick lime or calcium carbide.

(c) *Vacuum oven*.—Should be connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm. or less of mercury and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. A concentrated sulfuric acid gas drying bottle is connected with the oven for admitting dry air for releasing the vacuum.

(d) *Mercury manometer*.—Used to indicate the pressure of the partial vacuum.

DETERMINATION.

*Liquid Eggs*: Weigh accurately about 5 grams of the well mixed sample in the covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after room temperature has been attained. Remove the cover and drive off most of the water on the steam bath. Replace the cover and continue as directed.

**Powdered Dried Eggs:** Weigh accurately about 2 grams of the well mixed sample in the covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after room temperature is attained. Proceed as follows:

(Both liquid and powdered dried eggs)

Loosen the cover (do not remove) and heat at 98°–100°C. to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm. or less of mercury. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temperature is attained. Report the weight of the egg residue as total solids.

This vacuum method is considered the standard or umpire method for determining total solids in liquid eggs and powdered dried eggs.

(3) That a study be made during the coming year of a rapid method for determining total solids in eggs similar to the rapid routine method recommended for determining moisture in flour at 130°C. and at atmospheric pressure.

(4) That the method for determining ash in eggs be studied during the coming year. In this study attention should be given to the material of the ashing dish, as platinum may be injured by the high phosphorus content of the egg.

(5) That the following method for determining organic and ammoniacal nitrogen in powdered dried eggs and liquid eggs be adopted as official. (First action.)

#### *Organic and Ammoniacal Nitrogen.*

**For powdered dried eggs:** Transfer about 1 gram of well mixed sample accurately weighed to a 500 cc., or preferably 800 cc., Kjeldahl flask. Continue as directed below.

**For liquid eggs:** Weigh 2–3 grams of well mixed sample by difference into a 500 cc., or preferably an 800 cc., Kjeldahl flask. Continue as follows:

Determine the nitrogen by any one of the official methods<sup>1</sup>. (Complete digestion of the sample is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method.) Distil the ammonia into 30–50 cc. of 0.1 *N* standard acid

(6) That the following method for the determination of fat in liquid and powdered dried egg be adopted as tentative.

#### *Fat (Acid Hydrolysis Method).*

**For liquid eggs:** Weigh accurately by difference approximately 5 grams of well mixed sample into a 50 cc. beaker. Add 10 cc. of concentrated hydrochloric acid, mix well, set the beaker in a water bath held at 75°–80°C., and stir at frequent intervals for 15–25 minutes, or until the sample is sufficiently hydrolyzed to form a clear solution. Continue as directed below.

**For powdered dried eggs:** Weigh accurately 2 grams of well mixed sample into a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir to moisten all particles (the moistening of the sample with alcohol prevents lumping on addition of the acid). Add 10 cc. of hydrochloric acid (sp. gr. 1.125 or 25 + 13), mix well, set the beaker in a water bath held at 75°–80°C., and stir at frequent intervals for 15–25 minutes, or until the sample is sufficiently hydrolyzed to form a clear solution. Continue as follows:

<sup>1</sup> Assoc. Official Agr. Chemists, *Methods*, 1925, 6–9

(Both liquid and powdered dried eggs.)

Add 10 cc. of 95 per cent alcohol by volume and cool. Transfer the mixture to a Röhrig or Mojonner fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc. of ethyl ether in three portions and shake the mixture well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. Let stand until the ether layer is practically clear. Through a filter consisting of a pledget of cotton packed just firm enough in the stem of a funnel to allow free passage of the ether, draw off as much as possible of the ether-fat solution into a weighed 125 cc. beaker-flask containing some porcelain chips. Before weighing the beaker-flask, dry it in an oven at the temperature of boiling water and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc. of each ether. Shake well on the addition of each ether. Draw off the clear ether solutions through the filter into the same flask as before and wash the tip of the spigot, the funnel, and the end of the funnel stem with a small quantity of a mixture of the two ethers in equal parts free from suspended water. Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling water oven to constant weight (approximately 90 minutes). Remove the fat-flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

This method gives higher results than direct anhydrous ether extraction of the dried sample. The fat determined is probably essentially true fat, fatty acids, unsaponifiable matter, sterols, and coloring matter.

(6) That the following method for the determination of lipoids and lipid phosphoric acid ( $P_2O_5$ ) be adopted as a tentative method for liquid eggs and powdered dried eggs:

*Lipoids and Lipid Phosphoric Acid ( $P_2O_5$ ).*

*For liquid eggs:* Weigh accurately by difference approximately 10 grams of the well mixed sample into a 200 cc. nursing bottle, add 100 cc. of anhydrous ether, stopper with a softened cork, and shake vigorously. Add five 5 cc. portions of 95 per cent alcohol by volume and shake after each addition. The gradual addition of alcohol with shaking coagulates the proteins in a very fine state. Centrifugalize and decant the liquid off into a 250 cc. beaker containing some bits of broken porcelain. Wash the neck of the bottle with ether, and place the beaker with the fat solution on a steam bath. Continue as directed below:

*For powdered dried eggs:* Transfer about 2 grams of well mixed sample, accurately weighed, to a funnel having a pledget of cotton loosely placed in the stem. Wash with ether four or five times to extract most of the ether-soluble substances. Collect the washings in a 250 cc. beaker containing some bits of broken porcelain and place on a steam bath. Transfer the residue and cotton in the funnel to a small glass mortar and allow the ether to evaporate at room temperature. Add 2-3 grams of precipitated calcium carbonate to the egg residue, grind to a fine powder, and transfer all to a 200 cc. nursing bottle. Wash the mortar, pestle, funnel, and funnel-stem tip with ether and add washings to the original ether extract. Continue as follows:

(Both liquid and powdered dried eggs.)

Add 15 cc. of 95 per cent alcohol by volume to the egg residue in the bottle in such a way as to wash down any particles adhering to the sides and set in a water bath held at 70°-80°C. for 15 minutes. Shake occasionally with a rotary motion so as to moisten all particles with the alcohol. Cool, add 30 cc. of ethyl ether, stopper, shake for 5

minutes, centrifugalize to throw down suspended particles, and decant the liquid into the original 250 cc. beaker. Rinse the bottle neck with ether. Re-extract the residue with two successive 20 cc. portions of ethyl ether, shake 1 minute each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts to just dryness on the steam bath. Drive off any remaining apparent moisture on the sides of beaker by placing in a boiling water oven for about 5 minutes. Dissolve the dried extract in about 15 cc. of chloroform and filter the solution into a previously dried and weighed flat bottom platinum dish through a pledget of cotton packed in the stem of a funnel. Free any solid extract adhering to the beaker with a glass rod and transfer through the filter into the platinum dish by means of chloroform from a wash-bottle all soluble extract from the beaker bottom and sides. Finally wash the funnel and stem tip. (The filtrate should be perfectly clear.) Evaporate the chloroform on a steam bath (an electric fan may be used to hasten evaporation) and dry the dish and contents in a boiling water oven to constant weight (approximately 90 minutes). Weigh, and report the extract as lipoids.

*Lipoid Phosphoric Acid ( $P_2O_5$ ).*

Dissolve the lipoids in 10-15 cc. chloroform, add 10-20 cc. of 4 per cent alcoholic potassium hydroxide solution, evaporate to dryness on the steam bath, and char completely in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid (1 + 3) to make the solution slightly acid, and filter into a 100 cc. volumetric flask. Wash the filter and residue carefully, make up the filtrate to 100 cc., and determine the phosphoric acid by the official volumetric or gravimetric method. For the volumetric method pipet 20 cc. into a 250 cc. beaker, neutralize with dilute ammonium hydroxide (1 + 3), and then slightly acidify with dilute nitric acid (1 + 3). Set the beaker with the solution in a water bath held at 45°-50°C. and add 15 grams of ammonium nitrate. When the solution has reached the temperature of the bath, add sufficient ammonium molybdate solution, previously heated to 45°-50°C., to precipitate all the phosphates; stir; and heat for 30 minutes. Filter the precipitate on an asbestos mat in a Hirsch funnel, wash with cold water, and proceed according to the official volumetric method. Report as lipoid phosphoric acid ( $P_2O_5$ ).

(7) That study of the method for acidity of fat in eggs be continued during the coming year; that comparative results for acidity be obtained by the two methods of extraction discussed for this determination in this report; that trial be made to determine the acidity of the lipoids by titration of an aliquot of the alcohol-ether solution containing the lipoids before their darkening by heat in the method for lipoids, and that the remaining solution be used for the determination of the lipoids themselves; and that consideration be given to modifying the direct ether extraction method for the determination of acidity of fat so that it will harmonize with the other methods of egg analysis should this means of extraction prove the only satisfactory method.

(8) That the method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol and certain modifications of the method be studied further during the coming year. These modifications include the extraction of the water-soluble proteins with 1.2 per cent sodium chloride solution, the determination of the nitrogen of the precipitated albumin indirectly by difference as proposed by Associate Referee Palmer, and the

separation and washing of the precipitated albumin free from the mother liquid by centrifugalizing and decantation and the direct determination of nitrogen in the precipitate.

(9) That the study of the determination of zinc in eggs be continued during the coming year.

(10) That methods for the determination of unsaponifiable matter and sterols in egg products be studied during the coming year.

(11) That study be continued of the method for determining acid-soluble phosphoric acid in eggs; that consideration be given to the addition of the picric acid to the extraction mixture at the end of the half-hour period of shaking instead of at the beginning; that the phosphoric acid be determined by the volumetric method instead of by the gravimetric method; and that any other means of simplification of the method be considered.

## REPORT ON THE DETERMINATIONS OF THE ACIDITY OF THE FAT AND OF THE ACID-INSOLUBLE PHOSPHORIC ACID IN EGGS.

By H. I. MACOMBER (Food and Drug Inspection Station, New York, N. Y.), *Associate Referee*.

In the study of the method for the determination of the acidity of the fat in eggs, two methods of extraction were given consideration—the neutral extraction method for lipoids<sup>1</sup> and the direct extraction method<sup>2</sup>. While the former method measures more accurately the quantity of fat present, it was found that the final solution was too highly colored for satisfactory titration; it also takes more of the analyst's time than the direct method. For these two reasons, and because in the determination of acidity the total amount of fat is not important, the direct extraction method was chosen.

The method used is as follows:

### *Dried Eggs.*

#### REAGENTS.

(a) *Anhydrous ethyl ether*.—Prepare in the usual way from ordinary ethyl ether, or use absolute ether that has been kept over metallic sodium.

(b) *Neutral benzol*.—Use only benzol of the best quality obtainable. If the benzol available is not exactly neutral it should be refluxed for about 6 hours with about 0.1 of its volume of approximately normal sodium ethylate, distilled, and, if not yet neutral, washed three or four times with water. This is very important since slightly acid benzol will, during the titration of the ether extract, break down to form more acid with sufficient rapidity to affect the results quite seriously.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 93.

<sup>2</sup> *U. S. Dept. Agr. Bull.* 846, p. 90.

(C) *0.05 N sodium ethylate*.—Dissolve a piece of metallic sodium approximately 1 cc. in volume in 800 cc. of absolute alcohol. Titrate 10 cc. of 0.10 *N* hydrochloric acid with this solution and add the calculated amount of absolute alcohol to make the solution 0.05 normal. The normality factor of the solution should be determined the day it is used by titrating against 0.10 *N* hydrochloric acid.

#### DETERMINATION.

Weigh 2 grams of powdered dried egg into a tared aluminum dish about 2½ inches in diameter and dry in a vacuum of not less than 25 inches at 55°C. until there is no further loss in weight. Make the first weighing at the end of an hour and further weighings at intervals of one-half hour. (There is a slight gain in weight after the minimum has been reached.) Weigh to three decimal places.

Extract the dried residue with absolute ether, preferably in a Knorr apparatus, but if this is not available, in a Johnson extractor. Carefully transfer the egg powder to a 12.5 cm. hardened filter paper and roll into a cylinder that will fit rather snugly into the extraction tube, tucking in one side of the filter paper to prevent loss of material. (It is advisable to wrap the filter paper containing the egg in still another filter paper, which should be the 15 cm. size and the ordinary quantitative grade. No asbestos plug is needed in the extraction tube. If the extractor is working rapidly, 3 hours is sufficient to insure proper extraction.) Evaporate the ether from the extraction flask and dry the extract for one hour at 55°C. in a vacuum of not less than 25 inches. Weigh to three decimal places.

Dissolve the ether extract obtained as above in 50 cc. of the neutral benzol and add 2 drops of phenolphthalein indicator. Titrate with the 0.05 *N* sodium ethylate, expressing the results as cc. of 0.05 *N* sodium ethylate required to neutralize 1 gram of ether extract. The end point is reached when the yellow of the ether extract changes to an orange color.

#### *Liquid Eggs.*

The method for determining the acidity of fat in liquid eggs is the same as that used for dried eggs with the following exception:

Weigh a sample of approximately 5 grams accurately to three decimal places into a tared lead dish. Make the first weighing after drying for about 5 hours and at intervals of one-half hour thereafter.

In preparing the dried residue for extraction with ether, place the lead dish upon a fat-free filter paper, 12.5 or 15 cm. diameter, cut the sides of the dish through at four equidistant points, and flatten down. Place another fat-free filter paper on top of the lead dish, and roll the papers and dish into a cylinder that will fit into the extraction tube. (The ends of the cylinder must, of course, be turned in to prevent any of the material dropping into the extraction flask during extraction.)

#### COLLABORATIVE WORK.

Samples of both liquid and dried eggs were sent out to five collaborators. The dried eggs were from authentic samples, which were manufactured experimentally by the drum drying process in July, 1923. Each collaborator was sent four samples of these dried eggs, consisting of edible whole eggs, inedible whole eggs, edible yolk, and inedible yolk, the quality being that of the eggs before drying. The liquid eggs were broken at a New York egg dealer's plant by the associate referee and

were held in a sharp freezer until sent out to the collaborators. Four samples, consisting of the same four qualities as in the case of the dried eggs, were sent to each collaborator. The samples were shipped in a frozen condition, and precautions were taken to have them still frozen when received by the collaborator.

The results obtained and given in Table 1 show a fairly close agreement in the case of the dried eggs, and the differences between the edible and inedible eggs are quite marked. This striking variation is due to the fact that the eggs were stored at room temperature for more than a year after drying, and the inedible eggs continued to deteriorate while the edible ones showed very little change. In the case of the liquid eggs, it will be noticed that the results do not check as closely, especially those under "inedible whole eggs" and "inedible yolk".

The statement in regard to the end point in the titration of the fat was not included in the instructions as sent to collaborators, and it is thought that it will help to secure more uniform results from different analysts.

The following collaborators sent in results:

1. D. B. Scott, New York Station.
2. A. A. Boynton, Washington, D. C.
3. H. R. Smith, Baltimore Station.
4. L. A. Salinger, Savannah Station.
5. M. L. Hitchcock, Chicago Station.
6. H. I. Macomber.

TABLE 1.  
*Results of determination of acidity of fat in dried and liquid eggs.*  
(Results expressed as cc. of 0.05 N sodium ethylate per gram of fat.)

COLLABORATOR	DRIED EGGS				LIQUID EGGS			
	Whole		Yolk		Whole		Yolk	
	Edible	Inedible	Edible	Inedible	Edible	Inedible	Edible	Inedible
1	1.64	20.34	3.18	23.19	2.26	3.99	2.05	3.45
2	2.35	19.8	3.25	20.9	2.15	2.95	2.15	2.3
3	2.0	21.2	3.4	22.9	2.46	4.25	2.68	4.26
4	1.87	20.47	3.30	22.55	1.64	2.41	1.86	2.30
5	2.10	20.3	3.5	22.7	4.7	4.3	1.8	2.2
6	1.96	22.33	3.61	25.81	2.17	3.01	1.97	2.37

#### ACID-SOLUBLE PHOSPHORIC ACID IN EGGS.

The method originally used in the determination of the acid-soluble phosphoric acid in liquid eggs was the one described by Louis Pine, formerly of the New York Station of the Bureau of Chemistry, U. S.

Department of Agriculture, in his dissertation entitled, "A Study of the Acid-Soluble Phosphoric Acid in Eggs"<sup>1</sup>, and this method with some modifications was also used for dried eggs. It consisted of the extraction of the phosphoric acid with an aqueous solution of picric acid containing a small quantity of hydrochloric acid, the destruction of the organic matter by wet combustion, and the determination of the phosphoric acid by the gravimetric method<sup>2</sup>. This procedure was used on authentic samples of both liquid and dried eggs in experimental work at the New York Station for about two years. It was felt that the method as developed was rather long, and efforts were made to simplify it.

The original method called for shaking the solution after the addition of the picric acid by hand at 10 minute intervals for one hour. Equally good results have been obtained by placing in a shaking machine for one-half hour. Filtration of the picric acid solution was allowed to continue for three-quarters of an hour in the original method, and this time has been shortened by centrifuging the solution for a few minutes and decanting the filtrate.

Destruction of the organic matter by ashing with magnesium acetate, as described by Grossfeld<sup>3</sup>, was tried in place of the wet combustion method. While the results obtained were practically the same, the method is much longer and more complicated. The original method, therefore, was retained.

The determination of the phosphoric acid after the destruction of organic matter as originally made involved two precipitations, once as ammonium phosphomolybdate and the second time as magnesium ammonium phosphate. An effort was first made to weigh the ammonium phosphomolybdate precipitate, but it was found that uniform results could not be secured. Elimination of one step by precipitating the magnesium ammonium phosphate immediately after the destruction of the organic matter, as suggested by Scott<sup>4</sup>, was found to be practical and accurate.

The same samples that were sent to collaborators for the acidity of fat determinations were used for the determination of the acid-soluble phosphoric acid. The method used is as follows:

#### METHOD.

#### REAGENTS.

(a) *Magnesia mixture*.—See gravimetric method for the determination of phosphoric acid<sup>2</sup>.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 57.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 2

<sup>3</sup> *Chem. Ztg.*, 1920, 44: 285.

<sup>4</sup> *Standard Methods of Chemical Analysis*, 2nd ed., 1917, p. 315



(b) *1 per cent sodium chloride solution.*—Dissolve 10 grams of sodium chloride in 1 liter of water.

(c) *Ammonium hydroxide for washing.*—Dilute 250 cc. of concentrated ammonium hydroxide to 1 liter.

#### EXTRACTION OF LIQUID YOLK AND WHOLE EGG.

Weigh out into a sugar dish 50 grams of whole egg or 25 grams of yolk and carefully transfer to a 500 cc. centrifuge bottle with 200 cc. of hydrochloric acid solution containing 1 cc. of concentrated hydrochloric acid. Add 8 grams of picric acid, stopper the bottle with a rubber stopper, and place in a shaking machine for one-half hour. Centrifuge for several minutes and decant the clear liquid through a dry 24 cm. folded filter paper. The resulting solution should be absolutely clear. Centrifuging aids in rapid filtration and is more essential with whole eggs than with yolks. In the case of yolks that have been frozen it is important that the mixture of hydrochloric acid solution and yolk be shaken until all the lumps of yolk are dissolved; if this is not done, the results will be much too low.

#### DRIED YOLK AND WHOLE EGG.

Weigh out into a sugar dish 14 grams of whole egg or 12 grams of yolk and carefully transfer to a 500 cc. centrifuge bottle. Add 200 cc. of the 1 per cent sodium chloride solution, stopper the bottle, and shake till the egg and liquid are well mixed; then add 20 cc. of hydrochloric acid solution containing 1 cc. of concentrated hydrochloric acid and 8 grams of picric acid. Proceed as directed under "LIQUID YOLK AND WHOLE EGG".

#### *Destruction of Organic Matter.*

Pipet 150 cc. of the filtrate into a 500 cc. Kjeldahl flask; add four or five glass beads, 10 cc. of concentrated sulfuric acid, and 10 cc. of concentrated nitric acid; and boil until white fumes appear. Slowly add about 2 cc. of concentrated nitric acid and continue the boiling until white fumes reappear. Repeat this last step four times, then boil the mixture 10 minutes, and allow to cool. Add about 25 cc. of water and boil until any brown fumes are driven off.

#### *Estimation of Phosphoric Acid.*

Transfer the solution while still hot to a 400 cc. beaker (the tall form is preferable) and rinse the Kjeldahl flask four or five times with small quantities of hot water, being careful not to transfer the glass beads to the beaker. (A slight precipitate at this point may be disregarded. The total volume of the solution should not exceed 75 cc.) Add one or two drops of methyl red to the solution, make alkaline with concentrated ammonium hydroxide and nearly neutralize with dilute (1 : 3) hydrochloric acid.

After cooling, add 15 cc. of the magnesia mixture from a buret at the rate of two drops per second, rotating the beaker constantly. Allow to stand 15 minutes and add 15 cc. of concentrated ammonium hydroxide. Mix well and allow the solution to stand overnight. (A stirring rod should not be used during the precipitation of the magnesium ammonium phosphate because contact of the rod with the sides of the beaker causes the formation of crystals that are difficult to remove.

Filter through a Gooch crucible that has been washed with the dilute ammonium hydroxide, dried, ignited at intense red heat, and weighed. Rinse the beaker several times with the dilute ammonium hydroxide, carefully removing with a rubber policeman any crystals on the sides and bottom of the beaker. Wash the precipitate thor-

oughly with the dilute ammonium hydroxide, dry in a steam oven or at low heat in a gas muffle, add a few drops of saturated ammonium nitrate solution, heat at a low temperature until thoroughly dry, and ignite at intense red heat until the precipitate is white. It is sometimes necessary to repeat the addition of ammonium nitrate in order to obtain a white residue. Weigh as magnesium pyrophosphate and report results as milligrams of  $P_2O_5$  per 100 grams of sample on a moisture-free basis.

If desired, the magnesium ammonium phosphate may be filtered on an 11 cm. ashless filter, washed with the dilute ammonium hydroxide, and ignited in a tared porcelain crucible. After charring at a low temperature, add a few drops of concentrated nitric acid—sufficient to moisten the ash—dry on an electric hot-plate and ignite at intense red heat till residue is white. In calculating the total amount of liquid of which the 150 cc. is an aliquot part, be sure to include the amount of moisture in the eggs and in the picric acid. The moisture in the picric acid is determined by drying about an 8 gram sample over sulfuric acid in vacuo.

TABLE 2.

*Collaborative results on acid-soluble phosphoric acid in whole and yolk dried and liquid eggs.*

(Results expressed as milligrams  $P_2O_5$  per 100 grams of sample)

## DRIED EGGS

COLLABORATOR	WHOLE		YOLK	
	Edible	Inedible	Edible	Inedible
D. B. Scott	86.4	144.4	110.8	332.3
A. A. Boynton	132.0	179.2	143.6	435.1
H. R. Smith	155.0	206.7	135.2	384.0
L. A. Salinger	115.0	174.1	116.5	427.9
M. L. Hitchcock	105.2	130.9	122.6	395.2
H. I. Macomber	125.0	182.9	157.5	425.0

## LIQUID EGGS

D. B. Scott	89.4	97.6	94.5	98.7
A. A. Boynton	87.5	130.3	119.7	131.4
H. R. Smith				
L. A. Salinger	87.5	133.9	88.7	90.9
M. L. Hitchcock	64.5	105.3	19.1	93.8
H. I. Macomber	90.8	122.5	125.0	116.8

## COMMENTS ON RESULTS.

The results of the collaborators' work, as given in Table 2, check fairly well in the case of the dried eggs with a few exceptions, but the results in the case of the liquid eggs, especially the liquid yolks, are not very good. Of the six analysts, Boynton only reported results on the liquid yolks that are about what would be expected. The difficulty is probably due to the fact that the yolks were not sufficiently well mixed with the hydrochloric acid solution before the picric acid was added. After freezing, yolks are very thick, and unless a special effort is made to mix them with the acid solution they will remain in a lumpy condition even after

shaking in the machine, and the final results will be much too low. It will be noted, however, in every case except that of the liquid yolk, that the quantity of  $P_2O_5$  in the inedible product is much higher than in the edible one.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the method for the determination of acidity of fat, as described in this report, be given further study with the object of securing more concordant results.

(2) That the method for the determination of acid-soluble phosphoric acid, as described in this report, be given further study with the object of simplifying it and securing more uniform results. It has been suggested that it might be possible to precipitate the ammonium magnesium phosphate from the picric acid solution.

#### LIQUID AND FROZEN EGG PRODUCTS.

By MORRIS L. HITCHCOCK (Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation of the 1923 meeting, the Referee on Eggs and Egg Products appointed the writer to conduct a collaborative study of methods for the analysis of liquid and frozen egg products.

The work this year is essentially the adaptation of the important available methods<sup>2</sup> for the analysis of egg noodles to liquid and frozen egg products.

A preliminary investigation was made of the moisture or total solids determination for liquid eggs at the temperature of 55°C. and at the temperature of boiling water. To drive off the excess water, twenty 5 gram samples were weighed out in tared covered aluminum dishes and placed on the water bath. Ten of the dishes were placed in a vacuum oven at the temperature of boiling water. The vacuum gage read 25 inches, and the temperature registered 98°C. After these ten samples were completed, the remaining ten dishes were placed in the vacuum oven with the temperature around 55°C., the pressure being maintained as before. Aluminum dishes  $3\frac{5}{16}$  inches in diameter were used. The results are given in Table 1.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8, 273.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 84, 91

TABLE 1.  
*Percentage of moisture.*

AT THE TEMPERATURE OF BOILING WATER.					
SAMPLE NO	3½ HRS	4 HRS.	4½ HRS	5 HRS	5½ HRS.
1	74.06	74.08			
2	74.07	74.09			
3	74.00	74.01			
4	74.03	74.10			
5	74.10		74.17		
6	74.00		74.17		
7	74.09			74.24	
8	74.03			74.13	
9	74.05				74.28
10	74.02				74.28

AT THE TEMPERATURE OF 55°C.					
1	73.26				
2	73.47				
3		73.31			
4		73.55			
5			73.43		
6			73.47		
7				73.63	
8				73.48	
9					73.62
10					....

Another series of moisture determinations was made in aluminum dishes 2½ inches in diameter. The covers fitted down into the tops of the dishes and were left slightly tilted while in the oven. Two dishes containing 5 gram samples were placed in a vacuum oven with the temperature at 55°C. and the gage registering 25 inches. Two more were placed in another vacuum oven, the temperature being 95°C. and the gage reading 27 inches. The results are shown in Table 2.

TABLE 2.  
*Percentage of moisture.*

AT THE TEMPERATURE OF BOILING WATER				
	3½ HRS	4 HRS.	4½ HRS	5 HRS
1	73.75	73.84	73.88	73.91
2	73.66	73.79	73.83	73.86

AT THE TEMPERATURE OF 55°C				
1	71.02	71.70	71.97	72.19
2	70.05	71.16	71.59	71.75

Following this preliminary investigation, it was decided to submit to the collaborators the method on moisture determination that called for the temperature of boiling water in drying since this was shown to be the most satisfactory method for uniformity in operation and results.

The methods submitted to the collaborators are as follows:

## METHODS.

## PREPARATION OF SAMPLES.

(a) *Liquid Eggs*.—Secure representative container or containers. Mix the contents thoroughly and draw about a 300 gram sample for analysis. (A long handled dipper or ladle serves well to withdraw the sample.) Keep the sample hermetically sealed in a jar in a cool place. Report odor and appearance and examine for foreign matter.

(b) *Frozen Eggs*.—Secure representative container or containers. Examine contents as to odor and appearance and for foreign matter. (Condition of contents can best be ascertained by boring a hole to the center with an auger and noting the odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the borings made on the contents of each container. Borings should be taken midway between center and circumference from at least three widely separated parts of the container, and the depth of each boring should extend as nearly to the bottom of the can as possible.) Collect a sample of about 300 grams, seal hermetically in a jar, and keep in a cool place in a frozen state if possible. When ready for analysis, warm the samples in a bath held below 50°C. and mix well.

*Moisture—Total Solids*

## APPARATUS.

*Aluminum dish*.—Diameter about 55 mm., height about 15 mm., provided with inverted slip-in cover fitting tightly on inside.

## DETERMINATION.

Weigh a previously dried aluminum dish and cover, add approximately 5 grams of the well mixed sample, cover tightly, and reweigh. Drive off the excess water on the steam bath. Return cover to dish slightly tilted and dry in a vacuum at a pressure of not more than 5 inches at the temperature of boiling water, until there is no further loss in weight (approximately 5 hours). Press cover on dish tightly before removing from oven. Record oven temperature and pressure reading and report the loss in weight as moisture.

*Fat (Acid Hydrolysis Method).*

Weigh by difference approximately 5 grams of the well mixed sample into a 50 cc. beaker. Add 10 cc. of concentrated hydrochloric acid, mix well, immerse the beaker in a water bath held at 70°–80°C., and stir at frequent intervals for 15–25 minutes, or until the sample is sufficiently hydrolyzed to form a clear solution. Then proceed according to the method as published<sup>1</sup>.

*Lipoids and Lipoid Phosphoric Acid.*

Weigh by difference approximately 10 grams of the well mixed sample into a 200 cc. nursing bottle; add 100 cc. of any ether, stopper with a water-soaked cork, and shake the mixture well. Add five 5 cc. portions of 95 per cent alcohol by volume, shaking after each addition. The gradual addition of alcohol with shaking coagulates the proteins in a very fine state. Centrifugalize lightly and pour the ether solution off into a 250 cc. beaker containing some bits of broken porcelain. Wash the neck of the flask with ether. Place on a steam bath. Shake the residue in the bottle free by a rotary motion with 15 cc. of 70 per cent alcohol by volume, and place in a water bath at 70°–80°C. for 15 minutes with occasional shaking. Add 27 cc. of 95 per cent alcohol by volume and shake 2 minutes. Cool. Add 45 cc. of ether and shake 5 minutes.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 232.

Centrifugalize just sufficiently to throw the solid particles out of suspension, but not so as to pack the sample too firmly. Decant the alcohol-ether into the beaker containing the ether extract and rinse off the bottle neck with ether. Re-extract the egg residue with three 20 cc. portions of ether, shake 1 or 2 minutes each time, centrifugalize, and decant into the beaker containing the first extract. Proceed according to the method as published<sup>1</sup>.

*Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol.*

REAGENTS.

(a) *40 per cent alcohol by volume.*—Made by mixing 95 per cent alcohol by volume and water in proportions of 35 volumes of alcohol and 50 volumes of water.

(b) *Paper pulp.*—Shred some filter paper, macerate in hot water, and shake in a bottle with glass beads until well disintegrated. Remove water by filtering and suspend in 40 per cent alcohol by volume for use.

(c) *Asbestos.*—Prepare by igniting and rubbing through an 8-mesh sieve.

DETERMINATION.

Weigh by difference approximately 10 grams of well mixed sample into a 200 cc. nursing bottle. Add 10 cc. of dry ether, stopper with a water-soaked cork, and shake the mixture well. Centrifugalize lightly and filter the ether solution to catch any suspended solids. Add 50 cc. of ether twice more, shake, centrifugalize, and filter each time. Lay the bottle on its side and allow the excess ether to evaporate off at room temperature. Add 200 cc. of distilled water to the egg residue from a 200 cc. pipet, using a portion to wash into the bottle any decanted solids on the filter for the ether extract. Shake the bottle violently after the addition of about 50 cc. of water to avoid the formation of lumps, which later would prevent complete extraction. Shake the stoppered bottle in a shaking machine for 1 hour. The temperature of the water should not be much over 30°C. Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. The filtrate should be practically clear. Pipet 50 cc. of the filtrate into a 125 cc. beaker-flask or Erlenmeyer flask. Add 0.6 gram of sodium chloride and dissolve. Add a small quantity of finely divided filter paper pulp or asbestos and, with constant agitation, add 35 cc. of 95 per cent alcohol. Allow to stand overnight. Filter the mixture through a mat of paper pulp or asbestos in a Hirsch funnel, using light suction. Wash the flask and precipitate twice with the 40 per cent alcohol. Transfer the filter mat with precipitate to a Kjeldahl flask and determine the nitrogen by the Kjeldahl-Gunning-Arnold method, using 10 grams of sodium sulfate, 0.7 gram of yellow mercuric oxide, or its equivalent (0.65 gram) of metallic mercury, and 25 cc. of concentrated sulfuric acid. Use 30–50 cc. of 0.1 *N* acid to receive the ammonia of the distillate. Make a blank determination on the reagents and the filter paper pulp or asbestos.

*Organic and Ammoniacal Nitrogen.*

Weigh 2–3 grams of a well mixed sample by difference into a 500 cc., or preferably an 800 cc., Kjeldahl flask. Determine total nitrogen by one of the official methods<sup>2</sup>. (Complete digestion is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method.) Distil the ammonia into 30–50 cc. of 0.1 *N* standard acid.

The sample submitted to collaborators with these methods was prepared from fresh shell eggs. The eggs were broken, and the contents

<sup>1</sup> *Аннот. Official Agr. Chemists, Methods*, 1925, 233.

<sup>2</sup> *Ibid.*, 6–9.

were thoroughly mixed. A small quantity of thymol was added to prevent rapid decomposition. Portions of the sample were placed in small tin cans and sealed by paraffin. Each portion was shipped by express in an iced container.

The results of the collaborators are given in the table.

COLLABORATOR	MOISTURE	FAT (ACID HYDROLYSIS METHOD)	LIPOIDS	LIPOID P <sub>2</sub> O <sub>5</sub>	WATER- SOLUBLE PROTEIN- NITROGEN PRECIPITA- BLE BY 40 PER CENT ALCOHOL	TOTAL NITROGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
C. A. Greenleaf	74.12	11.46	12.89	0.35	..	1.97
J. L. Heid	74.11	11.45	12.81	0.35	.	1.94
Cincinnati, O.						
L. H. Bailey	74.22	11.09	12.87	0.38	1.17	2.05
Washington, D C.	74.18	10.91	12.79	0.36	1.01	1.97
M. L. Hitchcock	74.21	11.19	12.82	0.35	1.08	2.00
Chicago, Ill.	74.12	11.04	12.60	0.34	1.00	1.98
Average	74.16	11.19	12.80	0.36	1.07	1.99

#### COMMENTS BY COLLABORATORS.

*L. H. Bailey.*—Additional samples were dried in an electrically heated oven at atmospheric pressure and temperature at 130°C. for 1 hour. Loss of moisture was 74.12 and 73.90 per cent. These results indicate that a more rapid method may be used for drying egg than the present official method.

C. A. Greenleaf and J. L. Heid suggested that in the fat-by-acid-digestion method the sample be weighed directly into the extraction tube.

A review of the results reported will show that the methods are quite accurate and that different analysts are able to secure concordant results.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended that the methods—preparation of sample, moisture, organic and ammoniacal nitrogen, water-soluble protein-nitrogen precipitable by 40 per cent alcohol, fat, lipoids and lipoid phosphoric acid—be adopted as tentative methods for liquid and frozen egg products.

<sup>1</sup> For report of Sub-committee C and action by the association, see *This Journal*, 1925, 8: 273.

## REPORT ON METHODS FOR THE ANALYSIS OF DRIED EGGS.

By J. C. PALMER (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee*.

Last year's recommendation called for the study of methods for the analysis of dried eggs and the inclusion of methods already studied<sup>1</sup>. An attempt was made to apply the methods used in the analysis of egg noodles<sup>2</sup> to the analysis of whole dried egg.

The following determinations were given attention:

Taking and Preparation of Sample for Chemical Analysis

Moisture.

Fat (Acid Hydrolysis Method).

Lipoids and Lipoid Phosphoric Acid (Neutral Extraction Method).

Total Nitrogen.

Water-soluble Protein Nitrogen Precipitable by 40 per cent alcohol.

The sample used in connection with this work was one of powdered dried whole egg imported from China in 1922.

### METHOD.

#### *Taking of Sample.*

Sample powdered dried eggs that have been packed in barrels and boxes as follows: With the aid of a flour scoop, remove the top surface for a depth of 6 inches. Draw the sample and place in 3 quart Mason jars or other hermetically sealed containers.

#### *Preparation of Sample.*

Prepare the sample for chemical analysis as follows: Empty the contents of the three containers on a paper and return to a domestic flour sifter. Pass the product through the sifter and allow to fall into a cone shaped mass on the paper. Return to the sifter three times to insure complete mixing and breaking down of lumps. Fill the original jars and, if necessary, use one or more for the analysis.

#### *Moisture.*

An attempt was made to determine the maximum moisture obtainable under varying conditions of pressure, temperature, and time of heating by the tentative method<sup>3</sup>.

The results obtained are given in Table 1.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 84.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 231; *J. Assoc. Official Agr. Chemists*, 1923, 7: 86

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 231.



TABLE 1.

*Results of moisture determinations under varying conditions.*

TIME	PRESSURE 6 IN. TEMPERATURE 98°C.	PRESSURE 2.5 IN. TEMPERATURE 98°C.	PRESSURE 2.5 IN. TEMPERATURE 55°C.
hours	per cent	per cent	per cent
3½	6.19 6.21	6.36 6.35	5.87 5.91
4	6.22 6.26	6.29 6.29	5.89 5.94
4½	6.32 6.29	6.31 6.34	5.94 5.91
5	6.28 6.28	6.38 6.34	5.93 5.91
5½	6.28 6.30	6.30 6.38	5.99 5.98

The results show that the highest moisture percentage is obtained by heating in vacuum at the temperature of boiling water for a period of 5 hours and at a pressure not to exceed 2.5 inches.

*Fat (Acid Hydrolysis Method)<sup>1</sup>.*

This method, formulated for noodles, failed to give concordant results with dried egg. The temperature of digestion was therefore increased, and the quantities of sample varied until a maximum yield was obtained.

The results are given in Table 2.

TABLE 2.

*Results of fat determinations by acid hydrolysis method.*

SAMPLE	FAT	DIGESTION TEMPERATURE
grams	per cent	°C.
2	44.95 45.00 45.14 45.33	65-70 65-70 65-70 65-70
1.5	44.55 44.69 45.03 45.21	65-70 65-70 65-70 65-70
1	45.33 45.08	65-70 65-70
1	46.34 45.94	75-80 75-80
2	46.04 45.88	75-80 75-80
2	45.98 45.62	85-90 85-90

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1925, 232.

The results (Table 2) indicate that a digestion temperature of 75°–80°C. gives a maximum yield. Since the results obtained from 1 and 2 gram samples vary only slightly, it was thought best to recommend a 2 gram sample and thereby reduce any possible experimental error.

The method used varies from the published tentative method for alimentary pastes only in that a temperature of 75°–80°C. is recommended in place of 70°C. for the hydrolysis.

#### *Lipoids and Lipoid Phosphoric Acid<sup>1</sup>.*

The first part of this method, as formulated for noodles, can not be used for dried eggs, since it is necessary to remove as much as possible of the ether-soluble material before proceeding with the neutral extraction. This has been accomplished by placing the sample in a funnel having a plug of cotton loosely fitted in the stem. Four or five extractions with ethyl ether are sufficient to remove nearly all the ether-soluble substances. It is then necessary to reduce this powder to as fine a state of division as possible and to insure its coming into intimate contact, without lumping, with the extracting agents. Calcium carbonate, an inert material in this instance, when pulverized with the egg residue filled this requirement.

Experiments were carried out to determine whether or not the calcium carbonate was necessary and also what size sample produced the maximum yield of lipoids.

The results are shown in Table 3.

TABLE 3.  
*Results of determinations of lipoids.*

SAMPLE	CALCIUM CARBONATE USED	CALCIUM CARBONATE NOT USED
<i>grams</i>	<i>per cent</i>	<i>per cent</i>
5	48.96 48.53	
1	50.08 50.08	49.69 49.50
2	50.04 49.90	49.63 49.64
2	50.04 50.02	49.55 49.33

The results obtained (Table 3) indicate that the use of calcium carbonate is imperative and also that a one or two gram sample produces concordant results. The two gram sample was chosen because it tends to reduce the experimental error.

The method used is as follows:

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7, 93.

*Lipoids and Lipoid Phosphoric Acid.*

Place about 2 grams of sample, accurately weighed, in a funnel having a plug of cotton loosely placed in the stem. Wash with ethyl ether four or five times, or until practically all ether-soluble substances are extracted. Collect the washings in a 250 cc. beaker containing some bits of broken porcelain. Remove the residue and cotton to a small glass mortar. Allow the ether to evaporate and grind intimately with 2 or 3 grams of calcium carbonate. Transfer the fine powder and cotton to an 8 ounce nursing bottle and add 15 cc. of 70 per cent alcohol. Rinse the mortar, pestle, and funnel with ethyl ether and return the washings to the original beaker. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol and set in a water bath kept at 75°–80°C. Heat for 15 minutes with frequent mixing by the same rotary motion. Proceed according to the published tentative method<sup>1</sup>.

*Lipoid  $P_2O_5$ .*

Dissolve the lipoids in a little chloroform, add 20 cc. of 4 per cent alcoholic potassium hydroxide, evaporate to dryness on the steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid to make the solution slightly acid, and filter into a 100 cc. volumetric flask. Wash with water and neutralize with ammonium hydroxide. Acidify with nitric acid and make to volume. Pipet an aliquot (20 cc. equals 0.4 gram sample) into a 250 cc. beaker. Add 50 cc. of water and place in a water bath held at 60°–65°C. When the solution has reached the temperature of the surrounding bath, add 30 cc. of ammonium molybdate solution (acid added), stir, and let stand for 15 minutes. Filter the precipitate on an asbestos mat in a Hirsch funnel and proceed according to the official volumetric method for the determination of  $P_2O_5$ <sup>2</sup>. Report as lipid phosphoric acid ( $P_2O_5$ ).

*Total Nitrogen.*

An attempt was made to determine which of the three nitrogen methods—Gunning, Kjeldahl, or Kjeldahl-Gunning-Arnold modification—produced the maximum results with varying periods of digestion after clearing.

The results obtained are given in Table 4.

TABLE 4.  
*Results of total nitrogen determinations using three different methods.*

TIME	GUNNING METHOD	KJELDAHL METHOD	KJELDAHL-GUNNING- ARNOLD METHOD
<i>hours</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2	6.450 6.492	6.450 6.450	6.580 6.570
3	6.610 6.561	6.243 6.353	6.519 6.464

The Kjeldahl-Gunning-Arnold modification appears to produce the best results in the two hour digestion period.

The method as applied is as follows:

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 233.

<sup>2</sup> *Ibid.*, 3.

*Total Nitrogen.*

Determine total nitrogen as described under the official methods<sup>1</sup>. Use about 1 gram of sample, accurately weighed. (Complete digestion is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method, which requires about 2½ hours of digestion after clearing.) Distil the ammonia into approximately 50 cc. 0.1 N standard acid.

*Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol<sup>2</sup>.*

Two difficulties were encountered when this method was applied to powdered dried egg. The first, filtering the precipitated albumin, was overcome by adopting the indirect method as carried out in the casein determination of milk chocolate<sup>3</sup>. The nitrogen content was determined on the albumin solution before precipitation with 40 per cent alcohol and on the filtrate obtained after precipitation. The difference between the two nitrogen determinations gave the nitrogen content of the precipitate.

The next difficulty was to get concordant results in the water-soluble nitrogen. Varying quantities of sample and periods of shaking were resorted to, with the results given in Table 5.

TABLE 5.

*Results showing effect of varying quantity of sample and period of shaking.*

SAMPLE	PERIOD OF SHAKING	(A)	(B)	RATIO OF A . B
		WATER-SOLUBLE NITROGEN	ALCOHOL-PRECIPI- TABLE NITROGEN	
grams	hours	per cent	per cent	
1	½	3.553	2.303	1.54
1	½	3.637	2.443	1.49
2	1	3.897	2.844	1.37
2	1	3.897	2.925	1.33
2	1	3.301	2.362	1.40
2	1	3.301	2.332	1.41
2	2	3.399	2.511	1.35
2	2	3.732	2.732	1.36
2	2	3.718	2.760	1.35
2	2	3.245	2.329	1.39
2	2	3.827	2.925	1.31
2	2	3.982	2.957	1.34
2	2	3.897	2.872	1.35

These results (Table 5) seem to indicate that the quantity of albumin taken into solution varies directly with the period of shaking. All shaking was done at temperatures ranging from 25°–30°C. The resulting solutions were always colloidal in appearance, their turbidity varying directly with the period of shaking and with the nitrogen content. It was also observed that the greater the turbidity the more difficult it was to filter the solution. Ostwald and Fischer<sup>4</sup> state that albumin does not diffuse into distilled water, but will diffuse into weak salt solutions. Ostwald<sup>5</sup> shows that the solubility of albumin varies little when mixed

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1925, 6–9.

<sup>2</sup> J. Assoc. Official Agr. Chemists, 1923, 7, 86.

<sup>3</sup> Assoc. Official Agr. Chemists, Methods, 1925, 343.

<sup>4</sup> Theoretical and Applied Colloid Chemistry, 2nd ed., 1922, p. 46.

<sup>5</sup> Handbook of Colloid Chemistry, 2nd ed., 1919, p. 227.

with salt solutions of from 1.3–1.5 per cent. This idea, therefore, was applied to eliminate the turbidity in the water-soluble nitrogen solutions. The albumin formed a very clear solution in 1.2 per cent salt solution, and quite concordant results were obtained with a 2 gram sample, as shown in Table 6.

TABLE 6.  
*Results showing effect of using salt solution for infusion of albumin.*

PERIOD OF SHAKING	(A) WATER-SOLUBLE NITROGEN	(B) ALCOHOL-PRECIPTABLE NITROGEN	RATIO OF A : B
	per cent	per cent	
$\frac{1}{2}$ hours	3.455	2.458	1.41
$\frac{1}{2}$	3.483	2.458	1.42
$\frac{1}{2}$	3.530	2.628	1.35
$\frac{1}{2}$	3.528	2.598	1.36
1	3.827	2.788	1.37
1	3.592	2.468	1.45
1	3.455	2.530	1.37
1	3.469	2.519	1.38

The results (Table 6) indicate that the solution is complete after one-half hour shaking and also that the quantity of albumin going into solution as well as the degree of precipitation with 40 per cent alcohol is quite constant.

The method as revised and applied is as follows:

*Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol.*

Place approximately 2 grams of sample, accurately weighed, in an 8 ounce nursing bottle. Add 25 cc. of ethyl ether, cork the bottle, shake for several minutes, centrifugalize until the supernatant liquid is clear, and carefully decant off the ether solution, allowing none of the egg to be carried along. Extract three more times with 20 cc. portions of ether in the same manner. Wash off the neck of the bottle carefully with ether after each decantation to remove adhering fat. Dry the fat-free residue by aid of suction and reduce to a fine powder by working with a glass rod. Add slowly 200 cc. of 1.2 per cent salt solution and stir to avoid lumping. Stopper, and shake one-half hour in a mechanical shaker. The temperature of the solution should not be over 30°C. Centrifugalize to aid in filtering and filter through an asbestos pad in a Hirsch funnel, using light suction. Determine nitrogen in 50 cc. of the filtrate by the method described under total nitrogen<sup>1</sup>. Distil the ammonia over into 20 cc. of 0.1 *N* standard acid. Run blank determinations on the reagents used. Pipet 100 cc. of the above filtrate into a 200 cc. volumetric flask. Add 70 cc. of 95 per cent alcohol by volume. Mix carefully to avoid foaming, cool to room temperature, and make up to volume with 40 per cent alcohol (made by mixing 35 cc. of 95 per cent alcohol by volume and 50 cc. of water). Shake well and let stand overnight to allow complete precipitation of the all un in. Pipet off the supernatant solution and filter through an asbestos pad in a Hirsch funnel, using light suction. Determine nitrogen in 75 cc. of the filtrate by the method given for total nitrogen. Run blank determinations on the reagents used. Distil the ammonia over into 10 cc. of 0.1 *N* standard acid. Subtract the result obtained for the nitrogen contained in 75 cc. of the 40 per cent alcohol filtrate and multiplied by 4/3, from the nitrogen contained in 50 cc. of the water extract to obtain the water-soluble protein-nitrogen precipitated by 40 per cent alcohol.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 6–9.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the methods described in this report be submitted to collaborators for further study.

(2) That these methods be applied to dried albumin and yolk in powder and flake form and also dried whole egg in flake form.

(3) That additional methods be studied and perfected, such as organoleptic examination and preparation of sample of flaked dried egg products for chemical analysis.

## REPORT ON ZINC IN EGGS.

By WALTER E. KIRBY (U. S. Food and Drug Inspection Station, New York, N. Y.), *Associate Referee*.

The purpose of undertaking work as Associate Referee on Zinc in Eggs was to investigate the present method of analysis, and to improve it if possible or find a better one. This seemed to be desirable from the fact that the present method is long, depends upon the use of hydrogen sulfide with the attendant difficulty of filtering the somewhat colloidal sulfides, and does not always give very accurate results.

The plan of work was to carry on research with a method based on the work of Lundell and Bee<sup>2</sup> and Jamieson<sup>3</sup>, depending upon the gravimetric determination of zinc as precipitated mercury zinc thiocyanate. Among the advantages of this method are the ease in filtering the precipitate (due to its crystalline nature) and the small factor used in calculating the weight of precipitate to zinc, which also makes for accuracy.

The progress upon the work so far has been to establish the fact that the new method will give satisfactory results upon pure solutions of zinc. Efforts have also been made—but not completed—to adapt the new method to the ash obtained from dried eggs or to the acid digestion product of dried eggs.

## REPORT ON PRESERVATIVES.

By W. W. RANDALL (State Department of Health, Baltimore, Md.),  
*Referee*.

Upon learning of his appointment, the referee began a study of the recommendations of his predecessor. The possibilities suggested by Hortvet's paper on sublimation<sup>4</sup>, however, turned his attention to the problem

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 273

<sup>2</sup> *Trans. Am. Inst. Metals*, Sept., 1914, 146.

<sup>3</sup> *J. Am. Chem. Soc.*, 1918, 40: 1036.

<sup>4</sup> *J. Assoc. Official Agr. Chemists*, 1923, 6: 481.

of the purification of certain preservatives after extraction in impure condition from foodstuffs in accordance with the usual methods. Accordingly, plans were made for cooperative work upon the purification and determination of salicylic acid, benzoic acid, and saccharin. Delays in connection with the procurement of necessary apparatus and the necessity of acquiring a satisfactory technical experience in its use, coupled with the pressure of routine work, have made it impossible to present a report at this meeting.

#### RECOMMENDATION<sup>1</sup>.

The referee recommends that during the coming year a study be made of the ease and accuracy of the present official methods<sup>2</sup> for the determination in foodstuffs of salicylic acid, benzoic acid or benzoates, and saccharin, in comparison with modified methods involving the use of the sublimator.

#### REPORT ON COLORING MATTERS IN FOODS<sup>3</sup>.

By C. F. JABLONSKI (U. S. Food and Drug Inspection Station, New York, N. Y.), *Referee*.

Following the recommendation of the committee the referee herewith submits the following report on Coloring Matters in Food. As outlined by the committee, investigational studies were undertaken to extend the tests given under Section 15 of Chapter X, *Book of Methods*<sup>4</sup>, to include the coloring matters recently permitted. However, owing to adverse circumstances, collaborative work could not be arranged this year.

The coal tar dyes that have been added recently to the list of permitted food colors are:

1. Guinea green B, S & J No. 433.
2. Yellow OB (ortho, toluine azo beta naphthylamine).
3. Yellow AB (benzine azo beta naphthylamine).

#### GUINEA GREEN B, S & J No. 433.

This is a triphenyl methane type of dye, similar in reactions to light green SF yellowish S & J No. 435, the former being a disulfonate and the latter a trisulfonate of dimethyl dibenzyl diamido triphenyl carbinol. To differentiate guinea green B from light green SF yellowish it is ordinarily sufficient to extract a neutral aqueous solution of the dye containing about 5 per cent of salt with an equal portion of amyl alcohol, which will remove most of the guinea green B and only traces of light green SF yellowish. For a quantitative separation of these dyes the following

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 8: 274.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 125.

<sup>3</sup> Presented by J. A. Cummings

<sup>4</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 138, Chapter XI, 14, 146, 1925 ed.

modification, worked out by W. E. Mathewson, formerly of the Bureau of Chemistry, is preferable. This method consists of extracting a hydrochloric acid solution of the dyes with amyl alcohol and washing the amyl alcohol extract several times with an equal volume of a solution containing:

Sodium chloride	250	grams.
Sodium acetate	27.2	grams.
Acetic acid	24	cc.
Water sufficient to make	1	liter.

All the light green SF yellowish is extracted from the amyl alcohol by this salt and acid mixture, the guinea green B being left in the amyl alcohol. Orange I S & J No. 85, if present, will remain and interfere with the guinea green B in the amyl alcohol layer. To separate these latter dyes from each other the following procedure has given satisfactory results:

The aqueous solution of the guinea green B and orange I is extracted with several small portions of washed dichlorhydrin, which retains all the green and a small quantity of the orange dyes. The dichlorhydrin extracts are combined and washed with water, which process removes most of the orange I. To remove the last traces of orange I it is necessary to treat the dichlorhydrin layers with several 5 cc. portions of ammonia water (1 + 27) until the washings are colorless, whereupon all the orange I will have been removed. Finally, the dichlorhydrin extract is washed with 5 cc. of water to remove the traces of ammonia water. Then the dichlorhydrin is diluted with 5 volumes of carbon tetrachloride and the mixture extracted with several 10 cc. portions of 25 per cent alcohol, which will remove the guinea green B.

**YELLOW AB (BENZENE AZO BETA NAPHTHYLAMINE) AND YELLOW OB (ORTHO, TOLUENE AZO BETA NAPHTHYLAMINE).**

These dyes are not listed in Green's second edition of Schultz and Julius, "Organic Coloring Matters". Both are water insoluble, but soluble in alcohol, ether, chloroform, etc., giving a yellow solution. They are azo dyes and are reduced by stannous chloride, titanium trichloride, zinc dust, and other reducing agents. An alcoholic solution of any one of them, treated with strong hydrochloric acid, yields a red coloration. When either OB or AB is present, the following tests are of value:

Five cc. of a 0.001 per cent gasoline solution of the dye is shaken in a test tube with 5 cc. of a mixture of one part of 40 per cent formaldehyde and four parts of acetic anhydride. The coloring matter is extracted by the anhydride. Yellow AB forms a red solution in a few seconds, while yellow OB gives an orange solution under the same condition.

An alcoholic solution of AB, regardless of its strength, when treated with an equal volume of 84 per cent aqueous solution of citric acid, produces a rose-red coloration on standing, while yellow OB under the same condition does not change in color immediately, but gradually becomes colorless.



*The quantitative separation of yellow OB and AB.*

The method published by Mathewson<sup>1</sup> does not specify any fixed strength of acid for separating yellow AB and OB, and the use of sulfuric acid varying from 13–24 *N* is sometimes attended by unpleasant results. For that reason a simpler method was sought. Various color solvents and extractions with different normalities of acids and alkalies were experimented with. To report these would have no bearing upon the ultimate result. It may be of interest, however, to note in passing that phosphoric acid of 18 *N* strength gives fairly good results in extracting yellow AB and OB from low boiling (below 65°C.) petroleum ether. Also a 40 per cent (by volume) alcohol solution will extract small quantities of yellow AB but no yellow OB from low boiling petroleum ether.

The following method gave quantitative results:

## SOLUTIONS REQUIRED.

- (a) *Sodium hydroxide solution*.—400 grams made up to 1 liter with water.
- (b) *A mixture of alcohol and sulfuric acid*.—15 per cent alcohol by volume and sufficient sulfuric acid to make a 10 *N* solution.
- (c) *Ammonium acetate*.—100 cc. of a 50 per cent solution.
- (d) *Petroleum ether*.—Boiling point below 65°C.

In the following procedure, two separate sets of determinations were made, one with 4 mg. of yellow AB, and another with 4 mg. of yellow OB.

Four-thousandths gram of yellow AB was dissolved in 40 cc. of low boiling petroleum ether and transferred to a separatory funnel. To each of two additional separatory funnels 40 cc. portions of petroleum ether were added. To the first separatory funnel containing the dye dissolved in petroleum ether, 20 cc. of the 10 *N* acid-alcohol mixture was added and shaken vigorously for a short time. The acid-alcohol layer was then transferred to the second funnel and, after the second shaking, was transferred to the third funnel, from which after shaking it was transferred to a flask containing 150 cc. of water and 100 cc. of the sodium hydroxide solution. Three additional 20 cc. portions of acid-alcohol mixture were passed successively through the three separatory funnels, shaken in each case as before, and finally transferred to the flask containing the alkaline solution. A fifth 20 cc. portion was added to the second funnel and, after shaking, transferred to the third funnel, and then combined with the other four portions in the flask containing the alkaline solution. This operation removes practically all the yellow AB, but would leave in the petroleum ether any OB that might be present.

The three portions of petroleum ether were combined and washed twice with 50 cc. of water, once with 25 cc. of ammonium acetate solution, and finally with 50 cc. of water. After draining off the aqueous layer the petroleum ether was transferred to a casserole and evaporated on a steam bath at low temperature, the low temperature being necessary because the dye is somewhat volatile.

In the flask containing the acid-alcohol washings there was now a total volume of 350 cc. This solution was transferred in 50 cc. portions to a separatory funnel and extracted with 100 cc. of sulfuric ether, which removes all yellow AB present. If the acid extraction has been speedily performed, there is very little or none of the red phase (a form of decomposition) in the aqueous layer; consequently all the AB will be ex-

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12, 883

tracted. When all the color solution from the flask had been extracted by the ether, this was washed three or four times with 20 cc. of water; the ether was then transferred to a casserole and evaporated carefully on a steam bath at low temperature. After evaporation, it was taken up with several 10 cc. portions of 95 per cent alcohol and transferred to a 50 or 100 cc. volumetric flask, and the quantity of color present was estimated colorimetrically by means of a Schreiner colorimeter.

Standard solutions of AB and OB of 0.1 gram in 100 cc. of 95 per cent alcohol were used as the basis for comparison. It is imperative that the solution used for the colorimetric estimation be very dilute (about 0.001 per cent) in order to avoid errors in reading.

#### EXPERIMENTAL WORK.

In a liter Erlenmeyer flask containing 300 cc. of water, 285 cc. of concentrated sulfuric acid was added in small portions; this mixture was permitted to cool, after which 160 cc. of 95 per cent alcohol was added, thoroughly shaken, and transferred to a liter volumetric flask; after cooling it was made up with water to the mark. The titer of this acid, using methyl red indicator, was equivalent to 10.3 N.

The purity of the dyes was unquestioned, as they were samples of certified lots.

After the manipulation was carried out as explained above, the following results were obtained by the colorimetric method:

	per cent
Yellow AB—Petrol ether residue . . . . .	7.0
Re-extracted from acid washings . . . . .	91.0
Yellow OB—Petrol ether residue . . . . .	92.0
Re-extracted from acid washings . . . . .	3.0

Repeating these determinations several times gave nearly the same results. This indicates that the quantity of yellow OB washed out by the acid is approximately equivalent to the quantity of yellow AB retained by the petroleum ether. Several samples of various mixtures of yellow AB and yellow OB were prepared, and D. B. Scott was requested to separate them. The results are as follows:

	AMOUNT TAKEN			PERCENTAGE RECOVERED	
	OB (gram)	AB (gram)		OB	AB
Solution I . . . . .	0 0012	0 0028	in petroleum ether	100	88
Solution II . . . . .	0 0020	0 0020	in petroleum ether	101	86
Solution III . . . . .	0 0008	0 0032	in petroleum ether	103	75
Solution IV . . . . .	0 0030	0 0010	in petroleum ether	95	92

It was noted that in some instances the sulfuric ether extract of the AB after evaporation to dryness did not completely redissolve with alcohol and left a reddish residue in the casserole. This is probably a sodium sulfate lake of the AB. A further study to eliminate this error should be attempted.

If desired, the quantity of yellow AB may be checked by another method. The original color solution is diluted to a definite volume.

Exactly half is used for extraction. The yellow OB is compared colorimetrically against the original solution, the yellow AB being the difference.

Unfortunately, owing to lack of time, no studies were attempted upon other oil-soluble dyes, and the behavior of these in the presence of yellow OB and AB could not be established.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the separation of light green SF yellowish from guinea green B, the separation of orange I from guinea green B, and the separation of yellow AB from yellow OB be adopted as tentative methods, and that collaborative work be done on these.

(2) That additional work be done on the matter of separating yellow AB and yellow OB from other oil-soluble dyes.

(3) That further study be done on the "red phase" of yellow AB.

(4) That the changes and additions suggested in the revised chapter of coloring matters be adopted.

No report on metals in foods was made by the referee.

No report on arsenic was given by the associate referee. The recommendations as approved by Sub-committee C have been published<sup>2</sup>.

#### REPORT ON FRUITS AND FRUIT PRODUCTS.

By B. G. HARTMANN (Bureau of Chemistry, Washington, D. C.), *Referee*.

At the 1923 session of the association the following recommendations on the subject of fruits and fruit products were approved:

1. Study of methods for the determination of ash, alkalinity number, water-insoluble solids, alcohol precipitate, pectic acid, sulfur in ash and total sulfur, which were recommended for adoption as tentative methods by last year's referee.

2. Study of the official method for the determination of commercial glucose in jams, jellies, and preserves.

3. Study of methods for the determination of fruit acids in fruits and fruit products.

*Referring to Recommendation No. 1:* No changes in the technique of the methods are indicated. Comments relating to changes in the word-

<sup>1</sup> For report of Sub-committee C and action by the association, see *This Journal*, 1925, 8: 274.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 275.

ing of the text were submitted to the Chairman of the Committee on Editing Methods of Analysis. It is believed that the methods are suitable for the purposes for which they are intended and that they require no further attention at this time.

*Referring to Recommendation No. 2:* While searching for material that might serve as a basis for a critical study of the official method for determining commercial glucose in jams and jellies, the referee had access to an unpublished report<sup>1</sup> to the Bureau of Chemistry, United States Department of Agriculture, by C. P. Lathrop of the Food Control Laboratory of that Bureau, which bears directly upon the subject under consideration. The report records analyses of nine commercial glucoses and in the discussion calls particular attention to the uniform results obtained on the products when subjected to a polarization at 87°C. The report furthermore points out that the results obtained are in close agreement with those reported by Bryan in 1911<sup>2</sup>. In the light of the findings reported by Lathrop and Bryan, it may be assumed that the official method<sup>3</sup> is satisfactory when applied to glucose itself and furthermore that the method is applicable to the determination of commercial glucose in substances such as jams, jellies, and similar products. In making this statement it is assumed that preparations of commercial jams and jellies contain no substances that seriously disturb the optical rotation of commercial glucose at 87°C. It is believed, however, that in order to be assured of the correctness of this assumption the method should be tried on jams and jellies containing known quantities of glucose.

*Referring to Recommendation No. 3:* The association approved the appointment of E. K. Nelson as an Associate Referee on Fruits and Fruit Products, assigning to him the study of methods for determining fruit acids. As indicated in his report, the test to which the official method for the determination of tartaric acid<sup>4</sup> was subjected is a severe one, inasmuch as the quantity of tartaric acid contained in the solution submitted to the collaborators constituted only one-tenth of the total acid content. It is evident from the associate referee's report that under these conditions the official method is seriously in error and that the Kling method<sup>5</sup> is extremely accurate. Notwithstanding, the referee believes that the official method is convenient and suitable for most purposes and that with a slight modification it could be made more accurate. This belief is based upon the assumption that in the precipitate of the acid potassium tartrate organic acids that tend to raise the acidity above that due to potassium acid tartrate are occluded. It is believed that by reprecipitating the acid potassium tartrate the occluded acids may be

<sup>1</sup> NOTE.—This report has been rewritten and accepted by *This Journal* for publication as a contributed paper. It will be found on p. 714.

<sup>2</sup> *J. Franklin Inst.*, 1911, 172: 337.

<sup>3</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 188.

<sup>4</sup> *Ibid.*, 213.

<sup>5</sup> *Bull. soc. chim.*, 1910, 7: 567; 1912, 11: 886.

removed. However, it appears desirable to perform work during the next year regarding this point. The referee concurs in Nelson's recommendation to carry the Kling method as a tentative method.

In addition to the work bearing directly upon the three recommendations referred to above, a report is presented by H. J. Wichmann which treats of the determination of ash and solids in jams, jellies, and preserves. No provision was made by the association for conducting this work. The points discussed are of vital significance to the knowledge of the determination of ash and solids in jams, jellies, and preserves, and the recommendation made by Wichmann for further work seems desirable.

In his letter to the Chairman of the Committee on Recommendations of Referees, outlining plans for this year's work on fruits and fruit products, the referee indicated that he intended to study the lead acetate method for determining the acidity of fruit products. The lead acetate method is well known to chemists and has been repeatedly tried out by field analysts in connection with fruit work. The results obtained by the various chemists who have compared it with results obtained by direct titration with phenolphthalein have been unsatisfactory. It is recommended that further work on this method be discontinued for the present. The referee hopes to take up at some future time a study of the method for the purpose of determining its disturbing factors with a view to correcting the trouble.

In the course of his regular work several years ago the referee devised the following method for the determination of added water in white grape juice.

#### ADDED WATER IN GRAPE JUICE.

(APPLICABLE TO WHITE JUICES ONLY.)

Into a 2 ounce tincture bottle containing ten pieces of glass rod 15 mm. long and 5 mm. in diameter, introduce 50 cc. of the filtered juice at room temperature and approximately 1 gram of finely powdered cream of tartar. Allow to stand for a few minutes and then ascertain the exact temperature of the mixture by means of a thermometer graduated to 0.1°. Cork the bottle tightly and place it, neck downward, in a Mason jar. Fill the Mason jar with water at room temperature, screw top in firmly, place in a mechanical shaker, and shake for 1 hour. Again determine the exact temperature of the mixture and filter. To 200 cc. of recently boiled and cooled water in a porcelain casserole, add 3 cc. of phenolphthalein indicator and neutralize with 0.05 *N* sodium hydroxide solution. Add 10 cc. of the treated and filtered juice measured at 20°C. and titrate with the 0.05 *N* sodium hydroxide solution. Repeat the titration with 10 cc. of the original juice measured at 20°C. The two titrations should be made side by side in order to obtain the same shade of pink with the greatest possible accuracy. Calculate the percentage of added water contained in 100 cc. of grape juice, using the following formula:

$$x = \frac{0.0188 (b - a) - 0.095 - 0.025 (t^{\circ} - 25)}{0.006}, \text{ in which}$$

$x$  = percentage of added water;

$b$  = acidity of treated juice expressed in terms of cc. of 0.1  $N$  sodium hydroxide per 100 cc.;

$\sigma$  = acidity of original juice expressed in terms of cc. of 0.1  $N$  sodium hydroxide per 100 cc.; and

$t^\circ$  = mean temperature before and after shaking.

Commercially produced juices examined by this method show a small quantity, 1-3 per cent, of added water.

Although the method has not been offered for study by the association, the referee's personal experience with it has been satisfactory. It is believed that the method should be carried by the association as a tentative method. Attempts to make the method applicable to red grape juice have not been successful. In fact, it is believed that the method can not be made satisfactory for red grape juice since in its manufacture the process of heating causes a supersaturation of the product with cream of tartar.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That further study of the official method for the determination of commercial glucose in jams, jellies, and preserves be made.

(2) That the Kling method be adopted as a tentative method for the determination of tartaric acid.

(3) That a study of the determination of malic acid in the presence of citric and tartaric acids be undertaken.

(4) That methods for the determination of fruit ash in the case of fruit products containing added non-volatile ingredients like phosphoric acid, alum, or calcium salts be further studied.

(5) That the refractive index method for total solids in fruit products be further studied with a view to substituting it for the drying method.

(6) That the procedure for determining added water in white grape juice as described in this report be adopted as tentative.

(7) That the method for determining added water in grape juice be presented for collaborative work during the coming year.

(8) That the official method for tartaric acid be made the subject of further study for the purpose of improving its accuracy.

#### REPORT ON PECTIN IN JAMS, JELLIES, AND PRESERVES<sup>2</sup>.

By H. J. WICHMANN (U. S. Food and Drug Inspection Station,  
Denver, Colo.), *Associate Referee*.

Among several phases of fruit work that the associate referee desired to study this year was that of the accurate determination of fruit ash.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8 275.

<sup>2</sup> Presented by B. G. Hartmann

Although it is not directly associated with pectin, it is extremely important in the interpretation of fruit analyses. When jams, jellies, or preserves contain glucose, phosphoric acid, or alum an attempt must be made to estimate the fruit ash. The process is not simple, and the results obtained are at best only approximate. The estimation of fruit ash, where ash-bearing constituents have been added, is perhaps simplest in the case of added glucose. C. P. Lathrop<sup>1</sup>, formerly of the Bureau of Chemistry, found the ash content of present-day commercial glucose to be fairly constant, and he considered the figure obtained, 0.396 per cent, as being the average ash of dry glucose solids. In any given jam or jelly, therefore, the approximate proportion of the total ash contributed by the glucose can be calculated.

#### DIFFICULTY WHEN PHOSPHORIC ACID IS ADDED.

The problem is much more difficult in the case of those fruit products to which phosphoric acid (a common constituent of the cheaper jams, jellies, or preserves) has been added. A determination of the potassium oxide present in the fruit product might be one method of estimating the quantity of fruit ash, but it will be granted that estimating potassium oxide in such a mixture as a jelly is not easy. It was determined, therefore, to test the accuracy of another method devised in the Denver Station for this purpose.

A product containing phosphoric acid can not be ashed without loss of phosphoric pentoxide or potassium salts, or harm to platinum dishes. A known quantity of base, therefore, is added to the solution of fruit product to be ashed; it combines with the phosphoric acid, provides excess of base, and thus keeps the acid in the ortho form. Magnesium acetate was chosen because it readily burns to magnesium oxide at a low temperature, about dull redness. After ashing, phosphoric pentoxide plus the magnesium oxide determined in a blank is subtracted from the total ash. The difference is the fruit ash, or, in the case of glucose and fruit mixtures, fruit and glucose ash.

Soon after the study was started it was discovered that the magnesium oxide appeared to absorb either carbon dioxide or water, or both, quite rapidly from the atmosphere, and sometimes it was impossible to get a weight because the change was so rapid. This was remedied by covering the platinum dishes in which the ash determinations were made during cooling in the desiccators and while weighing. Since platinum covers were not available aluminum covers were substituted.

Materials containing phosphates are notoriously hard to ash. Although a clean ash was obtained by adding ammonium nitrate or nitric acid, these reagents obviously change the nature of the fruit ash. Various other schemes were tried to oxidize the carbon without at the same

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<sup>1</sup> See page 714.

time changing the character of the ash, but without success. As a last resort, the char was extracted with hot water in order to remove the soluble fruit ash as completely as possible. The residue was then moistened with concentrated nitric acid and ashed, usually without difficulty. A bright heat was employed to decompose the magnesium nitrate. The water extract was then added to the dish, evaporated to dryness, and heated to a low redness to avoid the volatilization of the potassium salts from the fruit ash. The method tentatively adopted may be described as follows:

#### METHOD.

Boil 300 grams of jam or preserve with 800 cc. of water for 1 hour. Cool, make up to 2000 cc., and filter. (A 15 per cent solution of jelly can, of course, be made up without boiling.) Place from 25–50 cc. of the filtrate in a weighed platinum dish with an aluminum cover. Add 10 cc. of a magnesium acetate solution that will yield a blank of approximately 0.2 gram of magnesium oxide per 10 cc., evaporate, and ash the solution. If a clean ash is obtained, remove the dish and aluminum cover to a desiccator and weigh as soon as cool. If it is impossible to get a clean ash, extract the char with hot water on an ashless filter. Ash the residue with repeated applications of small portions of concentrated nitric acid. Heat the ash to a cherry red to decompose the magnesium nitrate, then add the water extract, evaporate to dryness, and heat to a low red. Cover, cool, and weigh. Dissolve the ash in dilute nitric acid, make up to 100 cc., and determine phosphoric pentoxide in an aliquot by the official volumetric method<sup>1</sup>. Subtract from the total ash (fruit ash +  $P_2O_5$  + MgO + glucose ash, if glucose is present) the sum of total phosphoric acid minus that contributed by the fruit present (fruit estimated best by water-insoluble solids in the case of jams or preserves, and  $P_2O_5$  in the fruit from the average  $P_2O_5$  content of authentic samples of fruit) and the magnesium oxide obtained in the blank. The difference is the fruit ash plus the glucose ash, if glucose is present. A further calculation based on the average ash in glucose solids will give an approximate value for fruit ash.

#### ACCURACY OF METHOD.

The accuracy of this method was tested by determining the ash and phosphoric acid of various products (fruit juice, jelly, glucose), making definite solutions of them, adding phosphoric acid, and then proceeding according to the method. The results obtained are shown in Table 1.

The results shown in Table 1 indicate that the maximum errors of this method are about  $\pm 0.03$  gram per 100 cc. It is doubted whether more accurate results for fruit ash can be obtained by determining potassium oxide and calculating it to ash by means of a factor based on the average amount of potassium oxide in fruit ashes. Reactions at ashing temperature can not be controlled so readily as those at room or boiling temperature. The errors noted are, of course, larger than they would be if phosphoric acid had not been added, but keeping in mind the many steps in the operation and the uncertainty as to the exact reactions

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925 3



TABLE 1.  
*Determination of true ash in fruit products containing added phosphoric acid.*

	1	2	3	4	5	6	7	8
CHARACTER OF PRODUCT	ASH IN FRUIT PRODUCT	P <sub>2</sub> O <sub>5</sub> FRUIT PRODUCT	DILUTION	ASH IN DILUTED PRODUCT	ASH PLUS ADDED P <sub>2</sub> O <sub>5</sub>	ADDED P <sub>2</sub> O <sub>5</sub> (P <sub>2</sub> O <sub>5</sub> IN PRODUCT DEDUCTED FROM TOTAL P <sub>2</sub> O <sub>5</sub> )	TRUE ASH	ERROR DIFFERENCE BETWEEN COLUMNS 7 AND 8
	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>per cent</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>
Jelly made essentially from sucrose, pectin, and tartaric acid. Little or no fruit present.	0.156	0.012	none	0.156	0.410	0.230	0.180	+ .0240
Strawberry juice.....	0.590	0.0675	50	0.295	0.6556	0.3888	0.2668	-.0282
Blackberry and strawberry juice.....	0.478	0.036	50	0.239	0.6608	0.4399	0.2209	-.0181
Blackberry and strawberry juice. ....	0.478	0.036	10	0.0478	0.5200	0.4456	0.0744	+ .0266
Glucose solution, 15 per cent.....	0.0476	0.0041	none	0.0476	0.5320	0.5001	0.0319	-.0157
Strawberry juice.....	0.4300	0.0423	50	0.2150	0.5852	0.3700	0.2152	+ .0002
Blackberry juice.....	0.297	0.0236	50	0.1485	0.4264	0.3018	0.1246	-.0239

occurring in the muffle, it appears that the results are as good as can be expected under the circumstances.

It has been reported that some manufacturers add alum to jellies as an acidulant. This presents a new problem to the analyst; for example, when potassium alum is ashed with organic matter more or less sulfur is lost. To obtain the true ash under such circumstances it might be possible to subtract from the total ash the sulfur trioxide, aluminum oxide, and potassium oxide, making allowance for the potassium oxide present in the fruit. This, of course, would involve much work. It was thought advisable, therefore, to try the same method as for the addition of phosphoric acid for the following reasons: (1) The excess magnesium would probably hold the sulfur trioxide as well as the phosphorus pentoxide; (2) aluminum oxide could be determined in the total ash, calculated to potassium alum without water of crystallization, and this value subtracted from the total ash for the *true* fruit ash; and (3) a check could be obtained by determining the sulfur trioxide and calculating that to potassium alum. Accordingly, the method for determining the true ash of fruit products, to which phosphoric acid has been added, was modified to suit the addition of alum. The fruit solution, plus the magnesium acetate, ashes readily, and no difficulty was experienced. The sulfur trioxide was determined according to the official methods<sup>1</sup>. The total sulfur should be corrected for the sulfur that was present in the fruit and the glucose that might be present. Fresh fruits contain but little sulfur, and the correction in such cases can be neglected. If glucose is present, some allowance must be made for the sulfur in it. The sulfur trioxide, after correction, is calculated to potassium alum without water of crystallization. This figure is subtracted from total ash, and the difference is the true ash. The results can be checked by a determination of aluminum. The ordinary precipitation as aluminum hydroxide is not practicable in this case because of the phosphorus present in the fruit and the excess of magnesium salts. Aluminum was separated twice from the magnesium by the basic acetate separation. Since phosphorus was already present the aluminum was finally precipitated as the phosphate from weak acetic acid solution. Iron is usually present in fruits in such small quantity that it can be neglected. The precipitate of aluminum phosphate was washed, ignited, and weighed, and the weight of aluminum was calculated to water-free potassium alum. Necessarily, if another alum has been used, for example sodium alum, the proper factors must be used. The results obtained for the alum experiments are given in Table 2.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 211.

TABLE 2.  
Determination of true ash in fruit products containing added potassium alum.

	1	2	3	4	5	6	7	8	9	10	11
CHARACTER OF PRODUCT	ASH IN FRUIT PRODUCT	SO <sub>3</sub> IN FRUIT PRODUCT	DILUTION	ASH IN DILUTED PRODUCT	ASH PLUS ADDED ALUM	ADDED ALUM BASED ON SO <sub>3</sub> (SO <sub>3</sub> IN FRUIT PRODUCT DEDUCTED)	TRUE ASH BASED ON SO <sub>3</sub> DETER- MINATION	ERROR DIFFERENCE BETWEEN COLUMNS 7 AND 8	ADDED ALUM BASED ON AL <sub>2</sub> O <sub>3</sub> DETER- MINATION	TRUE ASH BASED ON AL <sub>2</sub> O <sub>3</sub> DETER- MINATION	ERROR DIFFERENCE BETWEEN COLUMNS 10 AND 11
	grams per 100 cc.	grams per 100 cc.	per cent	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
Strawberry juice . . . . .	0.4300	0.0080	50	0.2150	0.6928	0.4604	0.2324	+ .0174	. . . . .	. . . . .	. . . . .
Strawberry juice. . . . .	0.278	0.0068	50	0.1390	0.4948	0.3523	0.1425	+ .0035	0.3501	0.1447	+ .0057
Strawberry juice. . . . .	0.278	0.0068	12.5	0.0347	0.3490	0.2944	0.0546	+ .0199	0.2985	0.0505	+ .0158
Blackberry and strawberry juice	0.4450	0.0164	50	0.2225	0.6608	0.4486	0.2122	— .0103	. . . . .	. . . . .	. . . . .
Blackberry and strawberry juice	0.4450	0.0164	10	0.0445	0.5036	0.4625	0.0411	— .0034	. . . . .	. . . . .	. . . . .

## DISCUSSION OF RESULTS.

The results in Table 2 indicate that the order of accuracy for true ash, when alum is added to a fruit product, is about the same as in the case of added phosphoric acid. The error appears to be relatively the same whether there is much or little fruit ash present. That, of course, makes the percentage error much greater in a jelly made with sugar, added acid, and pectin and containing little or no fruit juice. These errors in the true ash determination will cause variations in the ratios based on ash, and proper allowance must be made in the interpretation of the analyses. It is realized that the results are not so numerous or so close to the true value as could be desired, but at present the chances of a better method and better results appear remote. While the method is not perfect, it appears to be the best available at the present time. It is recommended that the referee for next year endeavor to improve the details of the method, perhaps with reference to temperature and clean ashing, and then try them out with the aid of collaborators.

## TOTAL SOLIDS DETERMINATION.

The attention of the associate referee has been directed to errors occurring in the total solids determination, in the case of products containing high percentages of sucrose and organic acids. When sugar solutions containing acids are heated there is always more or less inversion, during which a molecule of water is added. Therefore, theoretically, the total solids should be increased by about 5 per cent of the amount inverted. Total solids are usually determined in fruit products by drying a portion first on the steam bath and then at 70°C. in the vacuum oven. This method presupposes that there is no inversion during the drying, but a surprising amount of inversion *does* take place. A 3.62 gram portion of a 50 per cent solution of sucrose containing by titration 0.90 per cent citric acid was weighed into an aluminum dish and thinned with water; the excess was evaporated on the steam bath and then dried in the vacuum oven at 70°C. After drying, 18 per cent of reducing sugar was found. Theoretically, the total solids found, 52.61 per cent, were too high by 0.90 of one per cent. In another experiment 300 grams of the same sucrose solution was made up to 2000 cc., 20 cc. was pipetted into an aluminum dish, and the excess liquid was evaporated on the steam bath and dried to constant weight in the vacuum oven at 70°C. The solids found were 52.30 per cent, with 23.3 per cent reducing sugar in the dried residue. Almost half of the total sucrose was inverted during the determination. There is then considerable inversion of sucrose in the act of drying when acid is present, and the errors inherent in the method can be quite appreciable. Under such circumstances the determination of non-sugar solids is absolutely useless. To obtain further

data, a solution was made of sucrose, tartaric acid, and water by the following formula:

	<i>grams</i>
Sucrose.....	50
Tartaric acid.....	1
Water.....	49
<hr/>	
Total.....	100

This solution contained 51 per cent of solids. Three to four grams was weighed into an aluminum dish and thinned with 15 cc. of water; the solution was spread out over the dish, and the excess water evaporated on the steam bath and then dried to constant weight in the vacuum oven at 70°C. The percentage of solids found was 52.66. The error therefore was +1.66 per cent. After drying, the residue was dissolved in water and made up to 100 cc., and reducing sugar was determined in a 25 cc. aliquot, the percentage found being 32.2. In other words, more than half of the sucrose present was inverted during the determination. The increase in solids due to the inversion was 5 per cent of 32.2, or 1.61 per cent. This checks remarkably close the plus error of 1.66 per cent found. These figures emphasize the facts to an unusual degree and demonstrate that the total solids determination by drying of a sucrose acid solution is entirely worthless. It follows that the non-sugar solids figures for such products as jams or jellies are likewise without value. As soon as the solution had been prepared, its refractive index of 1.4209, at 25.5°C., was obtained. This corresponds to a solids content of 50.90 per cent, according to Geerlig's table<sup>1</sup> for dry substance in sugar-house products. About 3 hours after preparation of the solution, the specific gravity of 1.2372, at 20/4, was found corresponding to a degree Brix of 51.37. Some inversion might have taken place in the 3 hours and thus caused the slightly higher result by the density over the refractive index method.

Still another solution was made by the following formula:

	<i>grams</i>
Sucrose.....	50.00
Dry commercial pectin . . . . .	0.75
Tartaric acid . . . . .	0.75
Water . . . . .	48.5
<hr/>	
Total . . . . .	100.00

This solution contained 51.5 per cent solids. The refractive index at 27°C. was 1.4218, which corresponds to 51.47 per cent solids. These results indicate that in the case of fruit products containing uninverted sucrose and acids the refractive index method for solids appears to be

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 343.

most accurate and convenient. In the case of jams or preserves, the total solids, of course, would be the sum of the water-soluble solids, as determined by the refractometer, and the water-insoluble solids. It is realized that these results are not sufficient to justify a recommendation that total solids in fruit products be determined by the refractive index method, but it is recommended that the referee for next year make a further study of the method for the purpose of making a definite recommendation.

#### SUMMARY.

The method devised at the Denver Station for determining fruit ash in the case of fruit products containing added non-volatile acidulants, like phosphoric acid and alum, has been tested and shown to have a maximum error of  $\pm 0.03$  gram per 100 grams. This appears to be the best that can be done under present conditions.

The drying method for determining total solids in fruit products containing sucrose and organic acids has been shown to be unreliable, due to the inversion of sucrose during the drying.

The few results obtained appear to indicate that the refractive index method for total solids is preferable to the drying method.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That methods for determining fruit ash in the case of fruit products containing added non-volatile ingredients, like phosphoric acid, alum, or calcium salts (recommended for increasing the efficiency of pectin) be further studied.

(2) That the refractive index method for total solids in fruit products be further studied, with the view to substituting it for the drying method.

#### REPORT ON FRUIT ACIDS.

By E. K. NELSON (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The Committee on Referees, at the 1923 meeting, recommended that methods for the determination of fruit acids in fruit and fruit products be studied and that an associate referee be appointed for this study.

The work along this line carried out this year comprises a study of the Auerbach<sup>2</sup> method for the determination of malic acid in the presence of other organic acids, and a collaborative study of the method of A. Kling<sup>3</sup> for the determination of tartaric acid.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8 275

<sup>2</sup> *Z. Nahr. Genussm.*, 1923, 46 97, 177.

<sup>3</sup> *Bull. Soc. Chim.*, 1910, (4) 7 567, 1912, 11 886

A careful study of the Auerbach method as applied to the determination of malic acid in the presence of equal or greater quantities of citric and tartaric acids convinced the associate referee that this method was totally unsuited for accurate work. Only about 70 per cent of the malic acid taken was recovered. The conversion of the acids into barium salts by treating their alcoholic solution with barium carbonate, as recommended by Auerbach, failed entirely, as barium carbonate was found to be insoluble in an alcoholic solution of the organic acids. This part of the method, of course, would have to be modified. Further, it was found that barium malate is incompletely soluble in water in the presence of barium citrate and barium tartrate, and this is the most serious defect in the method.

Further study is required to effect a satisfactory separation of these acids.

As the determination of tartaric acid by the official method<sup>1</sup> was found to give high results in the presence of considerable amounts of malic acid, a comparative study was made of the official method, a modification of the official method suggested by L. Chernoff<sup>2</sup>, and the A. Kling method.

The Chernoff modification consists in holding the solution containing the precipitated potassium acid tartrate at a temperature of 20°C. instead of in the ice box.

The Kling method is described as follows:

#### KLING METHOD.

##### REAGENTS.

(a) A solution of di-ammonium citrate containing 50 grams to the liter. (This is prepared by neutralizing 43.5 grams of crystalline citric acid with ammonia, evaporating to dryness on the water bath, and making up to 1 liter.)

(b) A solution of 20 grams of ammonium 1-tartrate, rigorously free from d-tartrate, to one liter. Five or 6 cc. of formalin is added to the solution as a preservative.

(c) Solution of calcium acetate made by dissolving 16 grams of calcium carbonate in 120 cc. of glacial acetic acid diluted with sufficient water, filtering, and making up to 1 liter.

(d) A solution of hydrochloric acid, 40 grams of the concentrated acid to 1 liter.

(e) A solution containing 5 grams of calcium carbonate dissolved in 20 grams of acetic acid with 100 grams of sodium acetate to 1 liter.

(f) A solution of potassium permanganate, 6.9745 grams per liter. This solution is standardized against a solution of pure tartaric acid of known titer. One cc. of the permanganate should correspond to nearly 0.005 gram of tartaric acid.

(g) A solution of oxalic acid containing 13.8793 grams per liter and titrated against the permanganate solution.

Carry out the standardization of the permanganate against tartaric acid just as prescribed for the determinations, namely, dilute the tartaric acid solution to 50-60 cc. with a solution of sulfuric acid, 10 per cent by volume. Heat nearly to boiling in a casserole and run the permanganate in slowly until it ceases to be decolorized on several minutes' standing.

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 213.

<sup>2</sup> Private communication.

Add the standard oxalic until the permanganate color has disappeared, or, if preferred, add a slight excess of oxalic solution and obtain the end point with permanganate. (It is to be noted that the final end point with tartaric acid is not obtained so easily as with oxalic acid, hence the oxalic end point is preferred. However, the tartaric acid must be completely oxidized, as the permanganate fades rather slowly towards the end of the titration.

#### DETERMINATION.

In order to effect the determination of tartaric acid, whether or not it is in presence of troublesome metals, to the solution brought to a volume of 150 cc., add 10–15 cc. of reagent (a), 25 cc. of (b), and 20 cc. of (c). Stir the solution well and allow to stand, with occasional stirring, for at least 4 hours; if interfering metals are present, allow to stand 12 hours.

Filter the precipitate on a Gooch crucible with a thin mat and wash six times with 10 cc. portions of water.

Dissolve the precipitate, after removal from the Gooch with the mat, with 20 cc. of (d) and wash Gooch thoroughly. Then adjust the volume of the solution to 150 cc., add 40–50 cc. of (e), and bring the temperature to 80°C. on the water bath. Cool the solution, stir, and allow to stand several hours. Refilter the precipitate on a Gooch and wash as before.

Remove to a casserole together with the asbestos, adding 50–60 cc. of warm sulfuric acid to 10 per cent volume strength and pouring through the Gooch in order to wash out any traces of the precipitate adhering to it.

Bring the solution in the casserole near the boiling point and add the standard permanganate (f) slowly so long as decolorized. When the color remains several minutes, run in a slight excess of standard oxalic acid solution (g) and add permanganate till a slight pink tinge is apparent.

The weight of tartaric acid represented by the permanganate consumed in oxidizing the tartaric acid divided by two, gives the weight of tartaric acid in the sample under examination.

The material submitted for collaborative work consisted of an aqueous solution containing 0.6 gram of tartaric acid, 3.0 grams of citric acid, 3.0 grams of malic acid, 12.5 grams of sugar, and 12.5 cc. of currant juice per 100 cc.

Analysis of this solution, containing so great an excess of malic and citric acids, imposes severe conditions on all three methods.

The results on tartaric acid reported by the collaborators, using 5 cc. and 25 cc. of the solution for analysis, are given in the table expressed as grams per 100 cc.

#### COMMENTS BY COLLABORATORS.

*E. O. Ealon.*—These results confirm my last year's work. I do not consider the Nelson modification of the Kling method troublesome or lengthy. It appears very satisfactory.

*A. M. Henry.*—This laboratory has no constant temperature room, and the determination was made at the room temperature. Any method requiring a constant temperature room is of very little use.

Cross does not seem to think that any of the methods are entirely satisfactory but that the Kling method, though rather complex, comes rather close to the truth.



ANALYST	OFFICIAL METHOD		CHERNOFF'S MODIFICATION		KLING METHOD	
	5 cc.	25 cc.	5 cc.	25 cc.	5 cc.	25 cc.
A. M. Henry Food & Drug Inspection Station Philadelphia, Pa.	0.405 0.405	0.363 0.363	0.225 0.135	0.279 0.321	0.565 0.570	0.565 0.564
V. B. Bonney Food & Drug Inspection Station Seattle, Wash.	0.74 0.76	0.74 0.74	0.62 0.68	0.80 0.77	0.60 0.60	0.56 0.55
E. O. Eaton Food & Drug Inspection Station San Francisco, Calif.	0.84 0.76	0.732 0.78	0.62 0.614	0.75 0.80	0.588 0.593	0.590 0.589
E. K. Nelson	0.78 0.74	0.90 0.90	0.68 0.66	0.88 0.84	0.63 0.66*	0.597 0.627*
L. J. Cross Department of Farms & Markets Albany, N. Y.	0.50	0.48	0.52	0.50	0.58	0.57

\* By determining Ca instead of titrating with permanganate.

It was noticed by the associate referee that when 25 cc. of this solution was used for the determination of tartaric acid by the official method, or its modification, 20 cc. of the wash solution was insufficient to remove excess malic and citric acids. Therefore the results are high. In determining tartaric acid by the official method emphasis must be laid on the necessity of using neutral potassium chloride in the wash solution. An experience that the writer had with a lot of potassium chloride having a strong alkaline reaction suggests that the low results reported by Henry may be due to the use of potassium chloride that was alkaline. Such a reagent in the wash solution would dissolve some potassium acid tartrate and vitiate the results.

The results obtained by the Kling method are uniformly satisfactory.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the Kling method be adopted as a tentative method for the determination of tartaric acid.

(2) That a study of the determination of malic acid in the presence of citric and tartaric acids be undertaken.

<sup>1</sup> For report of Sub-committee C and action by the association, see *This Journal*, 1925, 8, 275.

## REPORT ON CANNED FOODS.

By A. L. SULLIVAN (State Department of Health, Baltimore, Md.),  
*Referee.*

R. E. Doolittle submitted to the referee the manuscript of the chapter on Canned Vegetables for the revised methods of analysis of the association, prepared by the Committee on Editing Methods of Analysis. Certain changes were made. Paragraph 1, Physical Examination—Tentative, and paragraph 2, Preparation of Sample—Official, were enlarged and revised. The method for Moisture, paragraph 3, and that for Solids in Tomato Products, paragraph 13, were changed to require drying in vacuo at 70°C. for 4 hours. It is believed that the changes are justified, and therefore it is recommended that they be adopted. Experiments have shown that the method outlined for solids in tomato products, especially in canned tomatoes, gives results more accurate than those obtained by drying at 100°C., or by prolonged drying at 70°C. in vacuo. The action of the acid on the sugar tends to a gradual breaking down of the solids.

## EXAMINATION OF SPOILED CANNED FOODS—STRINGLESS BEANS.

In some of the previous meetings of the association W. D. Bigelow<sup>1</sup> has outlined the procedure for examining canned foods suspected of being spoiled, but in many instances the chemical and bacteriological examinations of such food are of value only when made by an expert. In recent months the attention of the referee has been called several times to flat sour stringless beans. Severe losses have come to canners by this type of spoilage. It seems that there is no practical method for separating the sound from the unsound goods without cutting the cans, although in some instances tapping will indicate suspicious cans.

The development of flat sours usually results in certain changes, such as increased acidity and presence of rod-shaped bacilli. As a supply of canned beans from a lot, a large proportion of which was flat sours, was available, it was decided to send specimens to several collaborators and to request them to make certain examinations. The specimens submitted represented good normal stringless beans and some flat sours. It was requested that the following examinations be made:

(1) If you have a suitable vacuum gage, determine the vacuum of each can and report the amount or absence of vacuum.

(2) Note the odor and taste of the canned vegetables and report findings. It is advisable to boil thoroughly before tasting spoiled canned food.

(3) Note the appearance of the liquid in the canned products and report whether clear or cloudy; also examine some of the liquid under the microscope and report whether any bacteria are found and, if so, the nature of the bacteria. It is desired that

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1920, 3, 1, 453; 1921, 5, 225

you determine if possible the number of bacteria present in the liquid, following the method given in paragraph 30, page 165 of the Official Methods of the A. O. A. C.<sup>1</sup> In reporting results, be sure and state the quantity of liquid used so that the number of bacteria can be estimated on a uniform basis.

(4) Titrate 25-50 cc. of the liquid from each can with 0.1 *N* alkali, using phenolphthalein as an indicator, and report the acidity as grams of lactic acid per 100 cc. of the juice.

After making the above examinations report figures obtained for each can and state whether or not the contents of each can are spoiled, and if so and it is possible, the nature of the spoilage.

#### *Examination of Cans.*

File a cross section through the crimped edge of the can a short distance from and on each side of the side seam of the can, using a three-cornered file. File through the outer sheet of tin plate between the two cross-section cuts and then press the end "hook" out of the seam. This will reveal the condition of the gasket and seam and indicate if the seam was properly made. Both the "factory" end and the "cannery" end of a can may be examined to locate the faulty double seaming. The factory end of the can is the one that has the smaller circles. The cannery end is the more important, and it is especially desirable to examine the seams of the cans, as many cases of spoilage have been traced to defective crimping, which results in slow leaks.

#### RESULTS OF EXAMINATIONS.

From the reports obtained and given in Tables 1 and 2, it will be observed that all collaborators were able to pick out flat sours, and that sour beans were found to have (1) a relatively high acidity, (2) large quantities of rod-shaped bacteria, and (3) little or no vacuum in the cans.

It should be noted that a number of cans in Group B were sound. It was of course impossible to foresee their condition, although the lot from which they came had been found to contain many flat sours.

The collaborators could not fully agree as to the cause of spoilage. Three believed it due to slow leakage and one to under-processing, although it was admitted that the seams were such that spoilage might be expected from leakage. Two experienced can men expressed the opinion that the seams at the cannery end were poor and that this caused slow leaks.

#### COMMENTS BY COLLABORATORS.

*National Cannery Association Laboratory.*—The vacuum was not determined on these cans, because it was desired to make a bacteriological examination and it was impossible, therefore, to take the vacuum without contaminating the contents.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920.

The cans were tapped, however, to determine the sound. Nos. 34, 32, 36, 38, 40, and 47 gave a dead sound; Nos. 43, 45, 49, 51, 53, and 55, a metallic sound, indicating a higher vacuum than those of the first group. Instead of tasting the contents the reaction to brom-cresol purple and brom-cresol green was determined. These indicators are commonly used in this laboratory to determine whether there has been a change from the normal pH of the product.

All the cultures taken from the sour cans gave negative results as to bacterial growth, showing that all the bacteria that had caused the souring were dead. From three of the other cans living bacteria were obtained, as follows: From No. 36 an aerobic spore growing only at 37°C.; from No. 40 a facultative acid-forming thermophile, and from No. 53 an obligate thermophile.

The contents of these cans were all in good condition; the bacteria were present, therefore, in a dormant form, and conditions were not favorable to their development.

An examination was made of the seams at both ends of the cans. The seams at the packer's ends of all the cans were very poor; those at the can company's ends were good, with the exception of No. 36, which showed a crack in the flange at this end.

We are unable to draw definite conclusions as to the cause of spoilage in cans Nos. 47, 49, 51, and 53. The seams at the packer's end are such that spoilage might be expected from leakage; however, the bacteriological indication is the opposite to this. In leaking cans the bacterial flora present is generally of a mixed character. In these four cans the microscopic examination shows bacteria of a similar type, and apparently in pure culture in each can.

It has been our experience that flat sours are in general due to under-processing. Taking these facts into consideration we would be more inclined to believe that this is a case of under-processing rather than leakage.

*P. L. Gowen.*—Although Nos. 15, 19, 21, 23, and 25 had possibly a slightly sour odor, I believe all of these cans with the exception of No. 25 could have been eaten without question. There was no distinct sour taste in the others.

Samples of normal and sour canned beans examined some months ago by me in cooperation with B. J. Howard gave the following results:

	ACIDITY— 0.1 N NaOH PER 100 cc.	APPROX- IMATE PH	BACTERIA— MILLION PER CC.
Normal cans—Average . . . . .	10 3	5 2	0
Min.—Max. . . . .	9 5–12 5	5 0–5 4	0–2.4
Sour cans—Average . . . . .	27 5	4 2	14 1
Min.—Max. . . . .	23 3–37 3	3 9–4 4	5 2–26 0

These data were obtained on 12 normal cans and 9 sour cans.

*L. C. Burroughs.*—Spoilage found in can No. 3 was probably due to a leak as there was no vacuum in the can.

*H. R. Smith.*—I conclude that cans 29, 31, and 35 were very obviously spoiled as indicated (1) by the absence of vacuum, (2) the odor, (3) the high acidity, and (4) the presence of rod-shaped bacteria. The contents of cans 24 and 25 had decomposed in perhaps a different manner, yielding a very bitter taste, although the acid, odor, and vacuum were normal. Cans 30, 33, 36, and 41 had little if any vacuum. Should not be used for food purposes. Cans 22, 28, and 39 showed no evidence of spoilage.

TABLE 1.  
Group A.—Consisted of 1924 pack stringless beans, in normal condition and put up by a high grade cannery.

COLLABORATOR AND CAN NUMBER	VACUUM	ACIDITY 0.1% NaOH PER 100 CC. OF LIQUOR	LACTIC ACID PER 100 CC.	BACTERIA PER CC. (ROD SHAPED BACILLA)	APPEARANCE OF LIQUID	ODOR	STERILITY	REACTION TO BROM-CRESOL PURPLE AND BROM-CRESOL GREEN
J. S. Bohart E. J. Cameron National Canners Association	<i>inches</i>	<i>cc.</i>	<i>grams</i>					
	32	8.55	0.077	None	O. K.	O. K.	Sterile	O. K.
	34	8.55	0.077	"	"	"	"	"
	36	8.66	0.078	"	"	"	Not sterile	"
	38	8.33	0.075	"	"	"	"	"
	40	9.00	0.081	"	"	"	"	"
P. L. Gowen Bureau of Chemistry Washington, D. C.	12	8.6	0.077	Negligible	Interior of tins bright	O. K.		Taste
	14	8.6	0.077	"	"	"	"	O. K.
	16	8.8	0.079	"	"	"	"	"
	18	9.2	0.083	"	"	"	"	"
	20	9.0	0.081	"	"	"	"	"
L. C. Burroughs State Department of Health Baltimore, Md.	2	9.36	0.084	3.2 million	Fairly clear	O. K.		O. K.
	4	9.79	0.088	"	"	"	"	"
	6	9.78	0.088	"	"	"	"	"
	8	9.79	0.088	"	Clear	"	"	"
	10	9.79	0.088	"	"	"	"	"
H. R. Smith Bureau of Chemistry Baltimore, Md.	Present	12.44	0.112	Negligible	Somewhat cloudy	O. K.		O. K.
	32	13.00	0.117	None	Clear	"	"	Bitter
	24	12.22	0.110	"	"	"	"	"
	26	12.0	0.108	"	"	"	"	O. K.
	28	13.22	0.119	"	"	"	"	"
Average Maximum Minimum	9.4 (8)	9.9 (20)	0.089	0		O. K.		O. K.
	12.0	13.2	0.117	3.2 million		"		"
	6.0	8.3	0.075			"		"

TABLE 2.  
Group B.—Consisted of cans taken from a shipment that had been found to contain a large percentage of flat souars.

COLLABORATOR AND CAN NUMBER	VACUUM	ACIDITY 0.1 N	LACTIC ACID 100 CC.	BACTERIA PER CC. (ROD SHAPED BACILLI)	APPEARANCE OF LIQUID	ODOR	STERILITY	REACTION TO BROM-CRESOL PURPLE AND BROM-CRESOL GREEN
National Canners Association			gram					
43		11.9	0.107	None	Slightly turbid	O. K.	Sterile	O. K.
45		12.5	0.113	"	Turbid	"	"	"
47		26.6	0.240	20 million	Slightly turbid	Off	"	Acid
49		33.6	0.303	50 million	Turbid	"	"	"
51		33.2	0.299	60 million	"	"	"	"
53		31.6	0.285	20 million	"	"	"	"
55		12.3	0.111	None	Slightly turbid	O. K.	Not sterile	O. K.
P. L. Gowen								
15	0.5	12.8	0.115	Negligible		Slightly sour	Interior of can marked etching	Possibly slightly sour
17	0	14.2	0.128	"		O. K.	Marked corrosion	Slightly sour
19	0	13.8	0.124	"		"	Marked etching	"
21	0	14.2	0.128	"		"	Marked etching	"
23	0	12.2	0.110	"		Distinctly sour	Marked corrosion	"
25	0	32.0	0.288	25 million		Slightly sour	Marked etching	Sour
27	0	13.6	0.122	Negligible		"	"	O. K.
L. C. Burroughs								
3	None	27.7	0.249	19.5	Fairly clear	Good		Slightly sour
5	4 1/2	13.2	0.119	"	"	"		Good
7	7 1/2	13.6	0.122	"	"	"		"
9	12 1/2	12.3	0.111	"	"	"		"
11	9 1/2	12.8	0.115	"	Clear	"		"
13	4 1/2	16.6	0.149	"	Fairly clear	"		"
A		35.2	0.317	44.0		Sour		Sour
B		29.4	0.265	"		"		"
C		34.3	0.309	"		"		"
D		33.0	0.297	16.		"		"
H. R. Smith								
29	None	38.0	0.342	Present	Clear	Abnormal		"
31	"	35.5	0.320	"	"	"		"
33	"	17.2	0.155	"	Cloudy	"		"
35	"	36.4	0.328	"	Clear	Normal		"
37	"	18.3	0.165	"	"	Abnormal		"
39	Present	16.0	0.144	"	"	Normal		"
41	None	18.0	0.162	"	Slightly cloudy	"		"
* { Average Maximum Minimum	0	32.8 (13) 38.0 26.6	0.295 0.342 0.24	31.8 million 44.0 16.0		Abnormal		Sour

\* For sour or spoiled cans.

In my opinion the spoilages noted were caused by a slight leak in the cans. The double seam of the factory end did not seem to be as tight as it should have been.

*Mr. Doolittle:* It is one of the pleasant duties of the presiding officer of this association to introduce the Honorary President, who really needs no introduction, to this audience. I take great pleasure in introducing Dr. Harvey W. Wiley.

#### ADDRESS BY DR. WILEY.

MR. PRESIDENT, MEMBERS OF THE ASSOCIATION, LADIES AND GENTLEMEN:

I have listened with great delight to the historical sketch of the beginnings of this association that has just been given by your president. I attended all the meetings that he mentioned with the exception of the first one in Washington. I must apologize to you for not having attended that one, but it was an unavoidable mistake; I did not know that it had been called. At that time I was a resident of a little known and little celebrated town called Lafayette on "the banks of the Wabash, far away". I was familiar with the sycamores, though I never had the experience of smelling the new-mown hay in the Wabash valley, because it does not grow there; it grows on the uplands. The poet was a little bit off his base when he described new-mown hay among the sycamores of the Wabash.

The notice of this meeting did not filter out that far; otherwise I should have been there, provided, of course, that the institution with which I was connected would have paid my expenses. But it was my good fortune to have attended the meetings in Boston and Atlanta and also the organization meeting in Philadelphia. I hoped that I might have here a picture of that organization meeting, but I haven't and now I hope that it does not fall into the hands of Mr. Haynes, because if it does that illustration will cause my arrest for illegal possession, as the picture represents me as holding high above my head a stein that probably contained a liquid that had more than one-half of one per cent of alcohol in it. At that time it was considered no offense to associate with a meeting of chemists a little sociability of this description, and it certainly couldn't have done any harm to the association because it has grown so big.

It is a fortunate thing that associations of this kind are not created, because if they were they would not have enough of the element of vitality that we can trace in the growth of this association. It is said that in the evolution of the human animal, when the germ is perhaps not more than an inch in length, little crevices are cut in the neck lead-

ing into the throat, showing that we are really akin to the fish and that in our early existence we are supposed to have had gills. So this association—and every association that has vitality—has an organism like an animal, or a human being, and we must look for its strength in its growth. And the picture of what this association has done, so vividly given by Dr. Doolittle, is a picture of the evolution and growth of an organization that is not supposed to be endowed with life.

As I listened to this address, it occurred to me that perhaps the most interesting thing I can say to you this morning is something in regard to the development of chemistry in this country. I am familiar with it by reason of my early birth—which was not my fault, for if I had had my way I would have been born about now, and I probably would have been born as honorary president and so have had a longer term in that position—but in my life, which looks very short as I look back over it, I have seen a great change in chemical science and the methods of teaching it. I want to review some of these methods.

I did not know anything about chemistry when I was a boy. It was an unknown science to the backwoodsman. The only real reference to chemistry that I had found was in an early book, somewhat respected but sometimes not so much respected as it should be, namely the Bible. I find there that one of the leading features of chemistry was fully comprehended. Baseball was also mentioned—and some of you know it has been featured here the past few weeks—because the Bible speaks of the beginning (big inning) and Rebecca was seen walking with the pitcher. I am sure McNeely had that in mind when he made the hit that brought Ruel in and won the world championship. The Bible says that when the Creator looked out he saw the world without form and void—in other words, a huge colloid. Those are simple allusions, and they came long before chemistry had developed to its present public interest.

In the beginning of the teaching of chemistry, in my recollection, the text book was the only method employed. To be sure, the professor of chemistry gave some experimental lectures that I thought were very fine, and as I look back upon them I know they were very fine, but the student had no access to the laboratory and without that he simply memorized, as a kind of a recitation, some of the facts that the teacher showed him in the book. That was the method employed not only in the small institutions but in the larger universities. I had occasion to look up the history of the injection of laboratory work into the colleges and universities of this country where the student had access to it and where it formed a part of his instruction. My attention was directed to this phase of the work particularly by a remark of President Eliot, which I saw in print, to the effect that his interest in chemistry was particularly delightful to him because he was appointed as an assistant to



Professor Cooke to help him prepare the lectures that he gave to the higher grade students and that there was where he learned chemistry, from the laboratory standpoint, by his actual preparation of experimental details for Professor Cooke. That was in 1851, when he was still an undergraduate at Harvard.

I wrote to several people asking if they could give me any idea as to when laboratory work was first introduced into teaching. I find some very valuable contributions. So far as we can ascertain laboratory instruction was first given in Princeton for a year or two before 1800. The students were taught the principles of chemistry by being put into the laboratory and set to work. And then I find that in William and Mary College, which is one of the oldest institutions in this country, James Madison, along in the year of 1807, gave some instruction by putting students to work in his laboratory.

Ann Arbor early advocated the principle of laboratory instruction. In 1844 the first professor of chemistry was appointed—that is the year of my birth and I remember that without much difficulty just now—Professor Douglas. He did not have any facilities then, but later he did have.

At Harvard, along about 1854 or 5, instruction in chemistry was given first by laboratory methods, and President Eliot doubtless had a great deal to do, although only a student at that time, in introducing that system into Harvard.

In the smaller colleges it came much later. I can speak of two of them—three in fact—with which I had something to do. One is Hanover, which is the oldest sectarian, or church, college. I do not like that word “sectarian” simply because a church established it, because what was taught there was by no means sectarian. That was the oldest church college in the state, having been founded in 1827. At first Hanover was an agricultural college and had a large farm. The students, many of whom were very poor in this world’s goods, worked in the fields for a part of their instruction, and agriculture was first taught in Indiana in that institution. From the beginning science was a distinct feature of the instruction of that institution, and as early as 1840 experimental lectures in chemistry were given there. When I entered Hanover in 1863 the teacher of chemistry was John Witherspoon Scott. He was not only a good Presbyterian pastor, but he was named for one of the signers of the Declaration of Independence. He was a very versatile man. He was not only a minister of the gospel, giving two good sermons every week, but he taught botany, physiology, zoology, and chemistry. Sometimes I was out late at night—always on good business, however—and when I would come home at two or three o’clock in the morning I would still see the light burning in his study. He was a fine teacher and an excellent sermonizer and the father-in-law of President Benjamin

Harrison. With a goose quill for a tube and a bottle for a retort that man made some of the best experimental illustrations I have seen anywhere. I was anxious to be an assistant to Dr. Scott but the chance never came to me. He made chemistry extremely interesting, as he did botany and the other studies that he taught. Now, those days have passed. We have no man that can teach all those sciences, even in a cursory way. He taught all the natural sciences, natural philosophy, as they called it.

My next teacher after I left Hanover was in the medical school. There I had for instructor Riley T. Brown. He was the state geologist of Indiana and he, like Dr. Scott, had a versatile mind. He taught well; geology was his specialty, and he was the first man to develop the mineral wealth of the state. Afterwards Professor Cox, the mineralogist, developed the coal resources of that state. Dr. Brown was a splendid lecturer and had a fine command of the English language. He was a preacher, too, preaching once every Sunday, sometimes twice. There I realized my ambition. Owing to the fine instruction that Dr. Scott had given me I soon got into the good graces of Dr. Brown and helped him prepare some of his lectures. It was a great opportunity for me and I soon began to take a great interest in the laboratory study.

So far as I know, the first laboratory desk was built in Indiana at what is now Butler College. I had already been to Harvard and taken my degree there and when I came back I was appointed professor of chemistry at Butler. Dr. Brown was a fine man, and he had an illustrious career. I had fine instruction under Brown; it was through that instruction that I was led to go on and get further instruction and later to abandon the classical field, devote myself to the study of chemistry, and take up practical work therein. In the autumn of 1873 I built 12 student desks in Butler College and put students to work there. Next year I went to Purdue. The field was open to me; there were no traditions to overcome. The first thing I did was to build 25 working desks in the laboratory. That laboratory has now expanded into a great building and has desks for several hundred men at a time.

So that is the second phase of teaching chemistry in this country; first, the textbook, second the textbook plus the laboratory. Then came the third phase, which included not only the textbook, which has not been abandoned, not only the laboratory where the methods of analysis, quantitative and qualitative, can be taught, but the synthetic methods by which the student can learn to make things not yet made. It is easy to tear down, but it is very difficult to build up. And there the third stage of chemistry, synthetic and research chemistry, was founded, and also the discovery of new principles and compounds. Before I go on to this, however, I want to say that Purdue University, now a great institution, as you know, had a very weak beginning. We started out with 37 boys, and

when we came to examine them we found that over half of them couldn't enter a high school; they were picked out so that out of the 37 we saved about 20 I believe. That was the nucleus of the Purdue of today. I put these boys—we had no girls there then—to making, not new things, but old things. I found that they were extremely interested in making something, and inorganic material for the most part. When the Centennial Exposition came along I conceived the idea of having an exhibit of things that the boys had made and I sent about 200 products made in the Purdue laboratory to Philadelphia in 1876. So far as I can find out that is the first exhibit of chemicals made by students at any exposition in this country. Now, you can find huge exhibits of this kind.

Then, finally, we come to the present system of teaching chemistry, with which you are all familiar. No one looking forward 50 years ago could possibly forecast in his imagination the advance in the teaching of this science. If he had had an imagination such as Thackeray or Dickens, or any poet, he couldn't have put into form what has already been accomplished and established in so far as the teaching of chemistry is concerned. So, among us, laboratories richly endowed, provided with all the paraphernalia and environment that any investigator could use, are rising on every hand; we have departments where science can be taught. Facilities for exploiting the unknown have been so increased that we must naturally expect in the near future a wonderful output in the realm of science. We think sometimes that nothing more can be discovered in chemical theory, but do you realize the fact that the theories I learned 60 years ago are now entirely swept away by recent investigations and discoveries so that if any of the great chemists who lived at that time, like Liebig, Silliman, or Hofmann, and men of that description, who were giants 50 years ago, were to pick up without explanation a modern work on chemistry they would not understand a word of the theory of it, so greatly and thoroughly have all the theories been changed. Theories may come and go, but the facts remain forever; the same laws, the same phenomena, the same mathematical proportions that characterized the chemistry of generations ago and of billions of years ago will remain and characterize the chemistry of a billion years hence, so when a theory is pushed aside no harm is done to the atom or to the nature of the thing. This is a fact in which we should rejoice, and the very facts of early chemistry, as taught to me, about its laws of combination remain unchanged with all of this changing of ideas with regard to the atom. Now, what reason have we to suppose that the present theory is going to continue? It explains the phenomena as they now appear. If any theory discredits the fact, the theory must give way and the fact must remain. So that this evolution is only the evolution of chemical theory.

I was taught that an atom is indestructible, but under the present theory that isn't true, although all the facts that attach themselves to the atom could be explained very well on that theory. Now we doubt whether it is matter or not; certainly parts of it, at least, are not matter but electrical charges. No one claims that an electrical charge is a material, but we must have material in order to have that charge. We find it in the nucleus—that is, the whole nature of the atom is in the nucleus. The particles and electrons that dance around this spot may pass away, but that does not change the nature of the nucleus. There must be some matter somewhere, and that is the only place to find it. So, the old theory has given way.

The principal thing I want to impress upon you is that if we do not have further progress in chemistry it is our fault, because we have been given every facility. Sometimes that is a great disadvantage. If you endow an institution too well you may introduce into it an element of decay. It is necessity that imparts vitality and progress. If no one ever got hungry, no one would ever start to produce food or increase its quantity. If hunger were done away with, agriculture would perish. If you furnish an investigator everything in the world that he wants so that he would never have to give any attention to his tools, you would destroy his mechanical skill and ability to discover or invent. How much would Berzelius have done, do you suppose, had he been put in one of these new-fangled laboratories? Why, Berzelius did his work in an old kitchen, and who ever did so much in the field of chemistry as Berzelius? To be sure, he had an unplowed field; he had an abundance of materials all around him. In southern Indiana when I was a boy you could go into any unplowed field, and you could gather up a basket of arrow-heads any time. That did not excite my idea of studying Indian technology; there were too many of them. Now you can go for hours and days in a field, freshly plowed, and not find one of them. It takes skill and energy if you have a restriction of the materials you are using, and I believe Berzelius would never have made the discoveries that he did if he had been placed in an environment where he would not have had to exercise his initiative and inventive ability.

Take Madame Curie and her husband, for instance. If they had been put into a laboratory with everything in it, would they have discovered anything? No, they would not have wanted to work.

And so, I say that we may destroy chemistry, to a certain extent, by too richly endowing it.

I have read in the histories of the great emperor of Rome, Marcus Aurelius, who was the finest pagan Christian that ever was. He went, as emperor, to visit Athens, which had fallen under the power of Rome, and he found a university there. He also found out that this university was a company of students, Aristotle or some other great Athenian at

the head, and that their university building was the steps of the Acropolis. The professor would stand in the shadow of one of the marble columns, and his students would sit at his feet. Marcus Aurelius was so impressed with the work of the university that he richly endowed it, and after it was endowed it was never heard of again. The stimulus for the teaching of philosophy was drowned in the flood of endowment. So, if any one is endowing an institution, beg him, for Heaven's sake, not to endow it too much.

I have briefly gone over, in these remarks, the progress in the past sixty years of my experience. After all, it is the chemist that uses the facilities he has that counts or the teacher of chemistry who is a human being—and many of them are. I discovered that teachers of chemistry at Harvard were human beings, much to my surprise; one of my most beloved teachers, many of you know, Charles Edward Munroe, is just as human as anyone, typically human. I want to tell you an incident to show that Harvard professors are human beings. While I was there John Tyndall gave a course of lectures at the Lowell Institute. At that time he was a great light—he is yet, though dead. I went to these lectures and at the close of the course Professor Cooke, head of the Harvard chemists, invited a few of the students to his house to meet Dr. Tyndall. Among those so favored was myself. I, a backwoods Hoosier, was invited to go to Professor Cooke's house and meet Dr. Tyndall, and that was the greatest event of my life up to that time. He was particularly interested in me because I came from what was called "the wild and woolly west". He took me to a corner and talked to me for about 30 minutes in regard to conditions in science in Indiana.

And Agassiz—I had the privilege of knowing him. Just think of the privilege of knowing and listening to Agassiz! That great man would take an egg and handle it so carefully that he would finally, before the end of the lecture, take all the shell off the egg and leave it unbroken, and talking all the time in such simple language that a child of eight could understand him.

And Professor Jackson, who has now retired, but is still alive. Munroe and Jackson are the only two teachers of chemistry in Harvard that I had that are now living. Professor Jackson, when I was last in Harvard to celebrate my fiftieth anniversary, invited me to come out to his home and I had a most delightful time. I used to think he was pretty cool, but he has warmed up now and is pretty human.

So, the personality of the teacher is just as great an expression as the science that he studies, and sometimes more so. We should appreciate our teachers more because in them lies one of the greatest sources of inspiration that can await us in after years. And I have felt this inspiration in the teachers that I have had—those old-fashioned teachers in Hanover and Harvard and especially in the medical college, and in Berlin

where I had the privilege of knowing men of the greatest standing in chemistry and physics, particularly Hofmann and Helmholtz. I esteem it one of the greatest privileges of my life that I have had the opportunity of knowing these men. It took me a long time to get my education because I earned it. From 1863, when I entered college, to 1879—a period of 16 years—I was trying to get all the education I could.

Last Saturday morning, which was my birthday, my oldest son asked me what hour of the day I was born. We have the hour at which he was born, but I said I did not know because the hour was not put down and my memory was a little vague on the subject, but I was sure it was as near as possible after midnight because my ambition always had been to begin early and finish late. And I have never finished my education. I am still educating, and I hope I will be as long as I live—and I do not know how long that is going to be—but I do want to live a long while and to live in the harness. When it pleases a kind Providence to take me out of the way of those who are coming after me (for that is what death means—the French say “Place au Jeune”, “Make way for the young”) I want it to be as near midnight as can be because I want to put in a full day's work the last day I live.

#### PRESENTATION OF BRONZE PLAQUE TO DR. WILEY.

The honorary president, Dr. Harvey W. Wiley, was the guest of honor of the Association of Official Agricultural Chemists at a dinner at the Franklin Square Hotel, in Washington, on the evening of October 21, 1924. The purpose of this gathering was to felicitate Dr. Wiley upon his eightieth birthday, which occurred on October 18, and to commemorate the fortieth anniversary of the founding of the association. Dr. Wiley was one of the twelve representative agricultural chemists present when the association was formed in Philadelphia, September 9, 1884. He was elected president of the association at the 1885 meeting and served for the customary period of one year. At the 1889 meeting he was made secretary-treasurer, and in this capacity for more than twenty years—until he retired from active membership in 1912—he served the association so well that upon retirement from active membership he was made honorary president for life. One of the features of each annual meeting of the association since that time has been the address delivered by its honorary president.

About 180 members of the association, and other friends of Dr. Wiley, attended the dinner. W. W. Skinner, the present secretary of the association, directed the after-dinner program in a delightful manner. No set speeches were made, but a few of those present were called upon and spoke on different phases of Dr. Wiley's life. Dr. Charles E. Munroe, who taught him in chemistry at Harvard, paid tribute to Dr. Wiley as

a student; Dr. B. B. Ross spoke of his service to the association; and Dr. C. A. Browne told of his work when Chief of the Bureau of Chemistry, of the U. S. Department of Agriculture. A number of telegrams and letters from friends that were unable to be present were read. After this came the most important part of the program for the evening. This was the presentation of a nine inch bronze plaque, a metallic reproduction of the original wax model of Dr. Wiley's profile used in the preparation of the medallion struck off in commemoration of the occasion. This medallion is two inches in diameter and, as shown in the cuts, bears upon its face the profile of Dr. Wiley with his name in full and degrees and on the back in an appropriate design the inscription, "Presented to Dr. Harvey W. Wiley on his eightieth birthday, October 18, 1924, at the fortieth anniversary of the Association of Official Agricultural Chemists, in recognition of his services to chemistry, agriculture, hygiene, and the public welfare". Replicas of the medallion had been prepared and were available to those who wished to have them either as mementoes or souvenirs. They may still be obtained from the secretary of the association.



The presentation was made by R. E. Doolittle, the president of the association. The honorary president was deeply touched by this evidence of the respect and esteem in which he is held by his friends, many of whom, at one time or another, had been associated with him in his work.

Following the presentation of the plaque, moving pictures of Dr. Wiley on his farm near Bluemont, Va., were shown. A number of cartoons that had appeared in the general and trade press at different times in his career and some questions and answers that might have come from the columns of "Good Housekeeping", prepared to illustrate the personality of the man and his highly developed sense of humor, were also thrown upon the screen. The occasion was one long to be remembered by those present.

R. W. BALCOM.

*Mr. Doolittle:* Just as Dr. Wiley appreciated those old teachers I think we have appreciated Dr. Wiley.

It has been customary for the Secretary of Agriculture to meet us at this point in our program, but unfortunately the Secretary has recently undergone an operation and is not able to appear. However, we have a very good substitute. I do not need to talk at length in introducing the representative of the Department of Agriculture at this meeting, but you are going to hear a good deal more of him, I believe, during the coming year. Dr. Wiley appropriately referred to him as not only the chemist but the Latin scholar. Dr. C. A. Browne will give us a few words now.

*C. A. Browne:* MR. PRESIDENT, DR. WILEY, AND LADIES AND GENTLEMEN: I have been injected into this program only by an accident, so my remarks to you are going to be very brief. It is customary at the annual meeting, as our president stated, that this association be addressed by the Secretary of Agriculture. I regret exceedingly that Secretary Wallace, who has spoken to you on previous occasions, was obliged to undergo an operation last Friday and it is impossible for him to be here this morning; on his behalf I have been asked to give you a word of greeting<sup>1</sup>.

It is needless for me as the representative of the Department of Agriculture to inform you that it always welcomes these meetings of this association. The Bureau of Chemistry has had particularly close contact with this association since the time of its organization in 1884, and it is hoped that this may always continue.

There are three things of outstanding importance connected with this occasion. The first is that last Saturday was the eightieth birthday of our honorary president, Dr. Harvey W. Wiley; the second is that this is the fortieth anniversary of the founding of the association itself; and the third is the completion of the new edition of our book of analytical methods. These are sufficient to stamp this meeting as one of most unusual character. The birthday of Dr. Wiley and the fortieth anniversary of the founding of our association are to be celebrated tonight in a very fitting way, and I shall confine my remarks only to a description of our revision of methods.

The revision of any methods is most chronophagous, if I may use a word that Dr. Wiley once coined. For you never know how much of the old to reject or how much of the new to include. The only safe way, I think, is to follow the course recommended by Pope when he said: "Be not the first by whom the new is tried, nor yet the last to lay the old aside". The issuance of our new book does not mean a complete cessation of our revisional work. This should be a continuous process. The coming edition of our new *Book of Methods* will be, I think, the most

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<sup>1</sup> Secretary Wallace passed away October 25, 1924.



complete and authoritative work upon the subject that has ever been compiled. We should not rest satisfied with this, however, but should always look for progress. In this connection, I should like to say just a word of appreciation of our president, Dr. Doolittle, who has had such an important part in the revision of the new edition of methods. I happen to know something of the labor that has gone into this work. It has been a most arduous task; he has given a great deal of time and effort to it, and I think that, as an association, we owe a great debt of gratitude both to him and to his staff of workers.

SECOND DAY.  
TUESDAY—AFTERNOON SESSION.

REPORT ON CEREAL FOODS.

By RAYMOND HERTWIG (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

It appeared to the general referee that his main service to the association was to develop the chapter on Cereal Foods in its *Book of Methods* so that eventually it will include all worthy known methods of analysis that may have value to any chemist interested in cereal analysis and cereal investigations and to develop these methods to their highest state of perfection.

Methods of analysis frequently fall into two general classes. The first class may be considered to include those methods giving most accurate results. Such methods are often tedious, costly, difficult of operation, and time-consuming. The chemist, however, must use these methods to obtain the most reliable results. They serve as standards by which are measured the merits of new methods and methods for ordinary practical application where simplicity, speed, and economy are of prime concern. The second class may be considered to include those methods whose chief merits are economy of time and cost, but whose limits of accuracy are somewhat wider than those from the so-called standard methods, although the results must be sufficiently accurate to enable their acceptance for all practical needs. The association's *Book of Methods* should first of all incorporate the standard methods of analysis. In addition, however, it is desirable to submit in juxtaposition to them the less accurate methods for determining the same substances, since they may be preferred owing to the demands of economy. Provisions should also be made for distinguishing these two types of methods.

Five associate referees were appointed in 1923 to study the following methods for the analysis of wheat flour: sampling, ash, moisture, and glutenin in flour, and chlorine in bleached flour.

The attention of the association is called to the following considerations of the methods studied and of the reports of the associate referees:

SAMPLING OF FLOUR AND THE PREPARATION OF THE  
SAMPLE FOR ANALYSIS.

The chief concern in sampling a product for analysis is to obtain a small quantity of the material of such composition that it will represent the average of the entire lot. Any subdivision of a homogeneous product may be taken as a representative sample. A product not homogeneous

throughout its mass must be made so in order that a small portion drawn for a sample may be strictly representative of the entire lot. In actual practice, most products are not homogeneous and, further, it is frequently impossible to make them so. The dependability of any physical and chemical examination of a product, no matter how accurate such examination may be, rests fundamentally upon the sample submitted for analysis. The most painstaking and accurate analysis is vitiated by a non-representative sample. Therefore, it is of prime importance that the procedure of sampling a product be given the most careful attention.

Flour in storage is packed in sacks containing from 6-140 pounds. A lot of sacked flour often containing many tons and representing a distinct type from a mill is not a homogeneous product. It is obvious, therefore, that the only practical means for sampling flour, in general, is by withdrawing portions from the sacks with sampling instruments.

Flour is a very hygroscopic material. Its moisture content varies with the humidity of its surroundings. Any interchange of moisture with the surroundings can occur only where the sack surfaces are exposed to the air. In flour in storage, the surfaces of the different sacks are irregularly exposed and consequently any moisture changes taking place in the individual sacks are unequally distributed about the flour mass and, in fact, can never take place in such a manner as to be equally distributed. The changes occur most rapidly in the outer layers of the flour mass. Thus the moisture changes are irregular both on the surface and throughout the flour mass of all the flour sacks. As such irregular variations can not be expressed mathematically, no sampling instrument can be devised with dimensions calculated from mathematical considerations that will enable one to withdraw a representative sample from a sack of flour stored under the conditions mentioned.

It is clearly apparent that a representative sample of flour in storage can only be approximated. No simple procedure can be recommended as the conditions encountered may differ widely. Experienced judgment must be called upon in each instance. Generally speaking, if large lots are under examination, it is better to draw a number of samples for separate analysis than attempt to get one composite representative sample that would be acceptable to those concerned in any transaction or legal action resulting from the analysis.

To sample a lot of flour in storage for the purpose of determining its moisture content at the time of packing at the mill or repacking by jobbers, it is advisable to withdraw cores of flour with a sampling instrument from the innermost parts of a sufficient number of sacks and to composite for analysis only those sections of the cores that come from parts farthest removed from any surfaces. To sample a lot of flour in storage for the purpose of obtaining as nearly as possible the average moisture content at the time of sampling it is probably advisable to

composite cores running diagonally through the flour mass of a sufficient number of individual sacks and extending between opposite ends of the sacks. Whether such a mode of sampling would procure a representative sample for this purpose has not been proved experimentally, and it is doubtful whether it can be proved practically for the reasons already mentioned.

The referee can report no actual sampling experiments. In keeping with what has been said, it is believed that only very general information and directions can be given in an association method for sampling flour. In the final recommendations of this report such general directions are given.

The associate referee, G. J. Morton of the San Francisco Station, Bureau of Chemistry, who was assigned the study of methods for sampling flour by the association, did not submit a report<sup>1</sup> to the general referee, so it is not known what work was undertaken or accomplished.

#### DETERMINATION OF MOISTURE IN FLOUR.

The present official method for determining moisture in flour<sup>2</sup> stipulates the drying of a sample "representing about 2 grams of dry material in a current of dry hydrogen or in vacuo at the temperature of boiling water to constant weight". Study of the vacuum method<sup>3</sup> has revealed that the loss in weight occurring when a flour sample is subjected to varying pressures and temperatures lower than those causing detectable decomposition is a function of both the temperature and pressure conditions. Ideally considered, the absolute moisture content of flour is a limit value that can only be approached, but not actually reached, under ordinary working conditions. Only under fixed conditions of pressure and temperature is the loss in weight a definite quantity, and vice versa, losses obtained under different conditions of pressure and temperature are unequal. The moisture content phase of a flour tends to be in equilibrium with the vapor pressure of the moisture of its surroundings. Therefore, to obtain uniform and duplicable results from a practical standpoint, a method must clearly stipulate certain arbitrarily fixed conditions of pressure and temperature. These conditions must be practically attainable and give results approximating the theoretical or absolute as closely as practice will allow, and the method must also stipulate certain other minor details of procedure necessary to avoid all controllable errors due to technique. It is the opinion of the referee that G. C. Spencer of the Bureau of Chemistry, acting as associate referee, has recommended a method that fulfils essentially the desired requirements for a working method. The revised description is given in the recommendations.

<sup>1</sup> Received too late for presentation. Published on page 680.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 161

<sup>3</sup> *J. Ind. Eng. Chem.*, 1920, 12, 40.

The associate referee found that the official method referred to previously<sup>1</sup> for the determination of moisture in flour in a current of dry hydrogen at 100°C. yields results much lower than those obtained by drying at 100°C. and at a reduced pressure of 25 mm. of mercury. This is to be expected as it is in accordance with the preceding observations. As results obtained by these two methods can not be the same, it is incompatible to have both for this determination in flour.

The vacuum method has all the merits of accuracy but not those of time and cost economy. In accordance with the plan given in the first part of this report, the associate referee devised a rapid method for determining moisture in flour capable of yielding results closely approximating those of the standard vacuum method. This rapid method should serve well in mill laboratories, for routine Governmental regulatory work, for the usual analyses in commercial laboratories, and in ordinary investigational work. This method is described in the final recommendations of this report as a routine method.

Both of the methods for moisture in flour discussed in this report determine the definite loss in weight a flour sample undergoes under certain definite conditions and the weight losses are constant when the conditions are as specified and constant. This fact makes the methods practical and usable for all considerations except the determination of the absolute moisture content of flour. Former methods are not practical because they do not always yield the same results. The loss in weight in the recommended vacuum method, which is calculated as moisture, has been shown so far as determinable, to be almost entirely water. The physical and chemical relationship of the moisture lost to the flour constituents is not considered in these methods, as present scientific knowledge of this relationship is not definite and conclusive. From available information, however, the loss in weight taken as moisture may be considered as the external water phase that is held by the surface attraction of the flour particles. The tremendous surface represented by a relatively small weight of flour sample, coupled with the great adsorptive power of flour for moisture, apparently is the factor that makes difficult the determination of moisture in flour. The molecules of water in direct contact with the flour surfaces are evidently removed with much greater difficulty than the more remote ones. Irrespective of how the moisture determined by these methods is related to the flour substances, it must be emphasized that the methods recommended are of value primarily because they always yield the same results under the conditions specified and because they give results as near the absolute as it is possible to attain.

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<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 161.

## DETERMINATION OF ASH.

Ash in flour is usually determined by igniting a sample portion in a furnace at a low red heat. This method is accurate if carried out under definite conditions, but it is time consuming. Higher temperatures cause fusing of the ash, losses due to volatilization, and consequently unsatisfactory results. It is desirable that some rapid method be devised for this much used and important determination.

L. H. Bailey and the referee found that the time of ashing can be considerably shortened by mixing the flour portion with a small quantity of glycerol before ashing. The burning of the glycerol-flour mixture produces a rather bulky charred mass with a greater exposed surface than flour burned alone, and thus promotes more rapid oxidation. The objection offered to the method is that the mixing of the glycerol with the sample portion and the burning off later require the attention of the analyst. Aside from the addition of the glycerol, the method is the same as the usual method of ashing. It is thought that the method may offer advantages under some circumstances, although it is not wholly satisfactory as a rapid substitute ashing method.

L. H. Bailey and the writer also have found that the addition of fine asbestos fiber to a flour very markedly shortens the ashing period. The asbestos serves to increase the exposed surface of the flour to the furnace atmosphere, to keep the charred mass bulky, and to allow free circulation of air through the char. Various types of flours were found to burn to a white ash in about 45 minutes at a temperature of about 575°C. For some unexplainable reason the results by this method are slightly lower than those by the regular ashing method, but excellent duplicating results are always obtained. Possibly some modification of the method will overcome this difference. The method certainly deserves further study because of the unusual rapidity of the oxidation and the simplicity of the procedure and because of the great saving of time that would result by using such a method where large numbers of flours are ashed, as in mill laboratories.

C. E. Mangels of the North Dakota Agricultural Experiment Station, acting as Associate Referee on Methods for Determining Ash, submitted to collaborative study a modification of the present official method of the association that was proposed by C. H. Bailey when acting as referee in 1922, and also the glycerol method. Mangels reports satisfactory collaborative results by the two methods and recommends the modified method for adoption as official. The associate referee also studied a method in which calcium acetate is added to the flour to prevent fusing of the ash and to make possible the use of higher temperatures. He recommends further study of the glycerol and calcium acetate methods.

## DETERMINATION OF GLUTENIN.

The determination of glutenin is attracting the attention of cereal chemists as offering a means of distinguishing between the baking qualities of different flours. Because of the apparent importance of this determination and because the present tentative indirect method gives low results, the association, at its 1923 meeting, appointed Paul F. Sharp of the Montana Agricultural Experiment Station as associate referee to study methods for this determination.

The quantitative separation of the protein glutenin from the other proteins and substances of a complex material like flour is an intricate and difficult problem, for the solution of which present knowledge does not offer an answer. During the past year Sharp has been carrying on excellent research work, but owing to the extensive experimentation necessary, he is unable at present to give more than a progress report.

This study of methods for glutenin determination necessitates a simultaneous study of methods for determining alcohol-soluble and salt-soluble proteins, for which the association now has tentative methods. Such thorough and detailed study should reveal means for improving these tentative methods.

DETERMINATION OF FAT (ACID HYDROLYSIS METHOD) AND LIPOIDS AND LIPOID PHOSPHORIC ACID ( $P_2O_5$ ).

Crude fat in flour by the present official method is determined by a direct extraction of the dried sample with dry ether. This method, according to Hertwig<sup>1</sup>, incompletely extracts the ether-soluble substances. However, another method devised by Hertwig in which the sample is digested with strong hydrochloric acid before extracting with ether extracts considerably more fat-like substances from flour than the direct ether extraction method. This acid hydrolysis method was studied collaboratively for alimentary pastes last year, was found satisfactory, and was adopted as a tentative method. Fat in flour and in alimentary pastes, by this method, is determined in exactly the same manner. Therefore, no collaborative study is necessary for its adoption as a tentative method in flour analysis. Since the method is to be given in the 1925 edition of the association's *Book of Methods*<sup>2</sup> under "Alimentary Pastes" it is only necessary to give reference to the method under "Wheat Flour" in a manner as indicated in the final recommendations of this report, which include an explanatory statement of the nature of the fats determined by this method.

It is believed that the acid hydrolysis method for fat determines essentially the true fats, fatty acids, unsaponifiable matter, and the sterols of

<sup>1</sup> *J. Assoc. Official Agr. Chem.*, 1923, 6: 508.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 232.

flour. Organic phosphorus substances such as lecithin are destroyed by the acid treatment as the extract is practically phosphorus-free. This determination of the organic phosphorus substances of flour is frequently of importance, especially in connection with chemical means for estimating the egg solids content of prepared wheat food products. It has been recognized for some time that the former method for extracting these organic fat-like phosphorus substances with hot alcohol has not been complete. A method for the determination of lipoids and lipid phosphoric acid ( $P_2O_5$ ) was devised by Hertwig<sup>1</sup> for this purpose and has been shown to extract more fat-like substances from flour and flour-egg mixtures in most instances<sup>2</sup> than any other known method. This method was adopted in 1923 as a tentative method for the analysis of alimentary pastes. Since the determination in the case of flour is exactly the same as for alimentary pastes, no collaborative work is necessary for its adoption as a tentative method for flour.

#### DETERMINATION OF WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

This determination was devised by Hertwig<sup>3</sup> to distinguish between egg alimentary pastes made with yolk and those made with whole egg and to aid in estimating the egg solids content. The method, it is believed, determines essentially the albumin of such products as noodles, eggs, and flours and very probably should be of value for distinguishing flour grades. The determination promises to be serviceable in flour analyses and should be included among the association methods for flour examination. It has already been studied collaboratively and adopted as a tentative method for alimentary pastes and its application to flour is exactly the same as for alimentary pastes.

#### DETERMINATION OF CHLORINE IN BLEACHED FLOUR.

It is desirable that a method for the determination of chlorine in bleached flours should give negative results for unbleached flours, should determine only that chlorine originating from the chlorine bleaching agent, should give quantitative duplicable results, and should determine no inorganic chlorides.

Associate Referee Armin Seidenberg studied various methods that seemed likely to fulfil these requirements. He was unable to submit the method developed by him to collaborative study, but he offers the method in his report as worthy of further study by the association. In the proposed method, the sample is first treated with warm 70 per cent alcohol. To this mixture are then added in the following order, with shaking after

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 91.

<sup>2</sup> *Ibid.*, 407.

<sup>3</sup> *Ibid.*, 84.



each addition, 95 per cent alcohol by volume, ethyl ether, and petroleum ether. The alcohol-ether solution is decanted off into a separatory funnel and washed with water to remove any inorganic chlorides. Any chlorine in the ether solution is determined and considered as chlorine coming from the bleaching agent.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the following directions for the taking and preparation of flour samples for analyses be adopted as tentative:

##### *Taking and Preparation of Samples.*

No simple rules can be given for the collection of a sample representative of the average of any particular lot of flour in storage as the conditions encountered may differ widely. Generally speaking, if large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample. It is important that the sampling be done in a systematic manner and that the same procedure be followed where circumstances permit. The number of samples to be drawn depends upon the quantity of the flour in question. Use that procedure of sampling which will obtain as nearly as possible a representative sample and be acceptable to those concerned in any particular transaction or legal action resulting from the analysis.

For the purpose of determining the moisture content at the time of packing the flour in sacks, sample by withdrawing cores of flour with a sampling instrument from the innermost parts of the flour mass of a sufficient number of sacks to assure a practically representative sample or samples of the flour lot under examination. Composite only those sections of the cores that come from parts farthest removed from any surfaces. For the purpose of determining the average moisture content of a lot of flour in storage at the time of sampling, withdraw cores running diagonally through the flour mass and extending between opposite ends of a sufficient number of sacks to assure a representative sample or samples.

Transfer the flour cores or sections of cores immediately to containers and seal tightly. Fill the sample containers not more than two-thirds full. Before removing portions of the sample for analysis, invert the container a sufficient number of times to make the contents homogeneous. Keep the sample tightly sealed at all times when not taking out portions for analysis.

(2) That further study be given to the principles involved in the method recommended for adoption as tentative for sampling flour with a view to the addition of any practical details to assist in the procedure. Consideration should be given to the type of sampler to be used and to the proportionate number of sacks to be sampled in lots of flour in storage. The collection of experimental data may be deemed advisable.

(3) That the present official vacuum method and the hydrogen drying method for the determination of moisture in flour be eliminated and the following umpire vacuum method be adopted as official for that determination:

<sup>1</sup> For report of Sub-committee C and action by the association, see *This Journal*, 1925, 8: 277.

*Moisture—Total Solids—Official Umpire Vacuum Method.*

APPARATUS.

(a) *Metal dish.*—Diameter about 55 mm., height about 15 mm., provided with an inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator.*—Should contain reignited quick lime or calcium carbide.

(c) *Vacuum oven.*—Should be connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm. or less of mercury and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. A concentrated sulfuric acid gas drying bottle is connected with the oven for admitting dry air for releasing the vacuum.

(d) *Mercury manometer.*—Used to indicate the pressure of the partial vacuum.

DETERMINATION.

Weigh accurately about 2 grams of well mixed sample in a covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature. Loosen the cover (do not remove) and heat at 98°–100°C. to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm. or less of mercury. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temperature is attained. Calculate the loss in weight as moisture.

(4) That the following routine method for determining moisture in flour be adopted as tentative and that study of the method be made during the coming year with a view to its adoption as an official method:

*Moisture—Total Solids—Tentative Routine Method.*

(For obtaining results closely approximating those of the official umpire vacuum method)

APPARATUS.

(a) *Aluminum dish and desiccator.*—As described for the vacuum method.

(b) *Oven.*—Should be capable of being maintained at 127°–133°C. and provided with an opening for ventilation.

(c) *Thermometer.*—To be placed with its bulb near the samples.

DETERMINATION.

Weigh accurately approximately 2 grams of the well mixed sample in a covered dish that has been dried previously at 127°–133°C., cooled in the desiccator, and weighed soon after attaining room temperature. Uncover the sample and dry the dish, cover, and contents in the oven at 127°–133°C. for 1 hour. Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temperature is attained. Calculate the loss in weight as moisture.

(5) That the following method for ashing flour be adopted as official and that the present official method be dropped (final action).

*Ash.*

Weigh 3–5 grams of the well mixed sample into a shallow, relatively broad ashing-dish, which has been ignited, cooled in a desiccator, and weighed soon after attaining room temperature. Incinerate in a furnace at approximately 550°C. (dull red) until a light gray ash results, or until no further loss in weight occurs. Cool in the desiccator and weigh soon after room temperature is attained.

Reignited quick lime or calcium carbide is a satisfactory drying agent for the desiccators.

(6) That the rapid methods for determining ash in flour mentioned in this report be studied during the coming year with a view to the development of a method especially adapted to routine work where time and economy are of great importance.

(7) That the study of methods for the determination of glutenin in flour be continued by the present associate referee and that in addition he be requested to include in his investigations any possible modifications of the present tentative methods for alcohol-soluble protein and protein soluble in 5 per cent potassium sulfate solution that his work may indicate as imperative.

(8) That the following methods for the examination of flour be adopted as tentative:

*Fat (Acid Hydrolysis Method).*

Determine as directed under this method for alimentary pastes<sup>1</sup>.

Higher results are obtained by this method than by direct ether extraction. The fat determined probably consists essentially of the true fats, fatty acids, unsaponifiable matter, and sterols. Such substances as lecithin are destroyed by the acid hydrolysis.

*Lipoids and Lipoid Phosphoric Acid ( $P_2O_5$ ).*

Determine as directed under this method for alimentary pastes<sup>2</sup>.

The extracted lipoids apparently contain all the ether-soluble substances in flour, true fats, fatty acids, lecithin and allied substances, unsaponifiable matter, sterols, waxes, coloring matter, etc. This method extracts many more ether-soluble substances than direct ether extraction.

(9) That the following method for the examination of flour be adopted as tentative:

*Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol.*

Determine as directed under this method for alimentary pastes<sup>3</sup>.

(10) That further study be given to the method developed by the associate referee for the determination of chlorine in bleached flours.

(11) That studies be made of methods for the analysis of bread and that in this connection effort be made to apply the association methods for wheat flour and alimentary pastes so far as is practicable and possible.

(12) That the determination of moisture in alimentary pastes be further studied with a view to the application of the two methods recommended for moisture in flour in this report.

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1925, 232.

<sup>2</sup> Ibid., 233.

<sup>3</sup> Ibid., 232.

## REPORT ON MOISTURE IN WHEAT FLOUR.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

In April, 1923, the writer was asked by the Bureau of Chemistry to undertake an investigation of moisture-in-flour methods for the purpose of establishing, if possible, a procedure that would at least be consistent with itself, reasonably accurate in its results, and applicable without the employment of expensive apparatus<sup>1</sup>.

The work was commenced by reviewing the methods of drying that had been in vogue in the Bureau of Chemistry for a number of years past, including the drying of flour in a stream of hydrogen gas.

Three typical samples of flour were purchased in sufficient quantities to last throughout the entire work. The following samples were selected: a hard wheat patent flour, a hard wheat clear flour, and a soft wheat straight flour. Each of these was placed at once in a wide-mouth, glass-stoppered bottle. It was believed that large samples thus preserved would maintain fairly constant moisture contents if well shaken each time that a sample was weighed out, and this has proved to be the case.

After confirming the unreliability of the methods<sup>2</sup> official at the time of this investigation, attention was next directed to the drying of flour in vacuo under standardized conditions. For one thing the pressure was carefully controlled so that it would not exceed 25 mm. of mercury throughout the drying. It was found that the pressure could be accurately controlled by a valve in a side tube through which air could be admitted to the vacuum line between the manometer and the pump. The pressure was read from a manometer instead of the usual vacuum gage.

It was found that excellent checking percentages could be obtained by holding the pressure at 1 inch and heating for 5 hours at the temperature of boiling water. It is to be emphasized, however, that the conditions of drying must be held as nearly constant as possible during the time of heating.

Aluminum dishes, cylindrical in shape, with closely fitting inside covers, 18 mm. high and 60 mm. in diameter, were used. For vacuum drying better results were obtained if the covers were allowed to rest loosely on the top of the dish. This lessened the probable chances of mechanical losses by air currents and also minimized the absorption of moisture when the dishes were being removed from the oven. The desiccators were provided with reignited quicklime in one case and calcium carbide in another. These are believed to be as effective for general purposes as sulfuric acid; they last much longer and do not have the disadvantages of a corrosive liquid in the desiccator. The vacuum method as recommended for use reads as follows:

<sup>1</sup> For original paper, see *J. Assoc. Official Agr. Chemists*, 1925, 8, 301.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 167

*Vacuum Method.*

Weigh accurately about 2 grams of the sample in a tared, covered dish. Loosen the cover and heat the dish and contents in a vacuum oven to 98°–100°C for 5 hours at a pressure not to exceed 25 mm. (1 inch) of mercury. Tighten the cover on the dish and cool for 20 minutes in a desiccator. Weigh and calculate the loss in weight as moisture.

This is recommended as a standard or referee method, but for routine work in mills or laboratories where many samples of flour must be assayed every day it has the following disadvantages: It takes too much time; it requires a suitable vacuum oven and pump with more or less elaborate fittings that require constant attention to keep them in repair; and it is expensive in installation and in upkeep.

For these reasons a substitute method was sought, one that would give satisfactory results for flour and at the same time obviate the disadvantages just enumerated.

The following procedure was devised after some experimentation.

*Substitute Method.*

Weigh accurately about 2 grams of the sample in a tared, covered dish. Remove the cover and heat the dish and contents in an oven at 130°C. for 1 hour. Replace the cover on the dish and cool in a desiccator for 20 minutes. Weigh and calculate the loss in weight as moisture.

This proposed new method is commended to the attention of all cereal chemists for the following reasons: The results are concordant and check closely with the results obtained by the standard vacuum method, and the time of operation is reduced from 5 hours to 1 hour.

In March of the present year two 4-ounce samples of clear and straight flours were sent to each of the nine collaborators that had signified their willingness to assist the associate referee. Their reports are given in the table.

Many of the results in the table will be seen to be lower than those given by the associate referee. This may be due to the loss in weight caused by evaporation of moisture from such small samples, although glass-stoppered bottles carefully sealed with paraffin were used. In the light of this year's experience it seems unfair to the method to send such small quantities of flour to collaborators. A sample weighing half a pound or even more would probably have made more concordant results possible. This naturally applies with equal force to flour samples intended for any kind of collaborative work, unless such results are reported on a moisture-free basis.

## COLLABORATORS' COMMENTS.

*C. B. Morrison.*—This whole subject of moisture determinations, especially in relation to the distribution of the various forms of moisture \* \* \* \* ought to be thoroughly gone over by a physical chemist in order to determine, if possible, just how the water exists in flour and how closely our methods determine the actual free moisture in distinction to that present in combination with the colloids as water of hydration. This would be a good job for the new Colloid Research Institute.

*J. B. Mudge, Jr.*—Suggest time of drying in proposed method be either extended or the temperature increased so that results be comparable to vacuum method.

*A. W. Meyer.*—All samples weighed into moisture dishes on April 11, covered with very tight-fitting covers and left in desiccator until ready to put into oven on successive days.

*M. J. Blish.*—Does not the size of the charge put into the oven have some effect on the length of time required for all the moisture to be driven off? In other words, if there were 20 samples in an air oven at 130°C. would the results at the end of 1 hour be the same as though there were only four samples?

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the present official methods for determining moisture in flour be dropped.

(2) That the methods described in this report be subjected to further collaborative study during the coming year.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8, 278.

Moisture results reported by collaborators.  
(a—Vacuum method; b—One hour at 130°C.)

COLLABORATORS	SAMPLE "A," HARD WHEAT CLEAR									SAMPLE "B," SOFT WHEAT STRAIGHT								
	1	2	3	4	5	6	7	8	Average	1	2	3	4	5	6	7	8	Average
L. H. Bailey Bureau of Chemistry Washington, D. C.	a 11.64 b 11.51	11.68 11.47	11.49 11.54	11.62 11.53					11.61 11.51	13.66 13.44	13.60 13.51	13.54 13.49						13.61 13.49
G. C. Spencer	a 11.65 b 11.63	11.66 11.66	11.72 11.69	11.70 11.71	11.71 11.65	11.71 11.66	11.61 11.62	11.60 11.63	11.67 11.65	14.03 14.07	14.09 14.11	14.10 14.11	14.13 14.08	14.13 14.08	14.06 14.05	14.04 14.03	14.07 14.03	14.07 14.07
M. J. Blish University of Nebraska Lincoln, Nebr.	a 11.56 b 11.55	11.53 11.47	11.51 11.50	11.56 11.43					11.54 11.48	13.88 13.75	13.78 13.73	13.78 13.69						13.82 13.73
Mary M. Brooke The Coby Baking Co. Washington, D. C.	a 11.06 b 11.20	11.16 11.23	11.49 11.27	11.50 11.23	11.39 11.31	11.48 11.31	11.58 11.46	11.43 11.37	11.38 11.29	13.31 13.42	13.76 13.50	13.81 13.47	13.70 13.52	13.70 13.52	13.69 13.58	13.61 13.58	13.21 13.56	13.55 13.51
E. N. Frank Washburn-Crosby Co. Minnesota, Minn.	a 11.70 b 11.53	11.72 11.61	11.74 11.55	11.85 11.62	11.82 11.35	11.59 11.51	11.78 11.50	11.62 11.49	11.72 11.49	14.01 13.82	14.03 13.81	14.17 13.81	13.98 13.81	14.02 13.43	14.02 13.58	14.03 13.53	14.33 13.56	14.07 13.69
Wm. C. Luckow American Institute of Baking 1135 Fullerton Ave., Chicago	a 11.38 b 11.38	11.46 11.39	11.01 11.40	11.03 11.40	11.19 11.47	11.20 11.47	11.13 11.26	11.15 11.40	11.19 11.39	13.72 13.66	13.77 13.69	13.23 13.72	13.25 13.80	13.45 13.74	13.44 13.70	13.36 13.65	13.44 13.59	13.45 13.69
A. W. Meyer W. E. Long Co., Chicago 155 N. Clark St., Chicago	a 11.59 b 11.68	11.54 11.61	11.59 11.64	11.62 11.63					11.58 11.64	13.97 13.88	13.91 13.86	13.90 14.02	13.92 14.09					13.92 13.96
J. B. Mudge, Jr. The Fleischmann Laboratories 158th St. and Mott Ave. New York	a 11.88 b 11.62	11.88 11.72	11.82 11.63	11.80 11.50					11.84 11.61	14.32 13.95	14.26 13.90	14.26 13.94	14.26 13.92					14.28 13.92
R. C. Sherwood University of Minnesota University Farm St. Paul, Minn.	a 11.56 b 11.16	11.53 11.21	11.44 11.26	11.46 11.23					11.49 11.21	13.81 13.50	13.85 13.48	13.85 13.69	13.83 13.66					13.84 13.53
a—Maximum Minimum Average b—Maximum Minimum Average									11.84 11.19 11.55 11.65 11.21 11.47									14.28 13.21 13.84 14.07 13.49 13.73

REPORT ON ASH IN CEREAL PRODUCTS<sup>1</sup>.

By C. E. MANGELS (Agricultural Experiment Station, Fargo, N. Dak.),  
*Associate Referee.*

The purpose of the work on ash this year has been to find a method that will give accurate results in a short time. The method now generally followed by cereal chemists (igniting in a muffle at low temperature) gives accurate results but requires too long a period for ashing.

The glycerol method suggested by Hertwig and Bailey<sup>2</sup> has been considered, and samples were sent out for collaborative work. This method has been subjected to collaborative study by the American Association of Cereal Chemists and reported by C. H. Bailey<sup>3</sup>. The tabulation of his results is included in this report as Table 1. In the writer's opinion the results obtained by Bailey do not warrant adoption of the Hertwig-Bailey method. Some collaborators have reported difficulty in preventing glycerol flour mixtures from frothing over the sides of the crucible.

For collaborative work the writer sent out two samples of flour—a patent and a first clear. The directions sent with samples follow:

## COLLABORATIVE WORK ON ASH METHODS—A. O. A. C. 1924.

*Method I.*

Ignite a crucible and when cooled, weigh, and rapidly weigh into it 5 grams of flour. Ignite in a muffle at approximately 550°C., taking care that no portion of the muffle becomes sufficiently hot to fuse the ash. A light gray, fluffy ash should result. Cool crucible and contents in a desiccator and weigh immediately after it reaches the temperature of the laboratory air.

*Method II. Hertwig-Bailey Method.*

Mix 5 grams of flour in the ashing dish with 10 cc. of a glycerol-alcohol solution made from equal volumes of each. Clean the mixing rod with a small piece of ashless filter paper. Ignite the paper with the sample over a free flame until the mass is charred. Transfer to a muffle and complete ashing at 575°C. Cool crucible and contents in a desiccator and weigh immediately after it reaches the temperature of the laboratory air.

Collaborators will please report time required for ashing by each method, and the referee will appreciate any comments on these methods that collaborators care to make.

Method I, the modified method proposed by C. H. Bailey in 1922, is the procedure generally followed by cereal chemists.

The results of collaborative work are given in Table 2. The results on patent flours show fairly good agreement between the two methods, but some collaborators obtained distinctly higher results by the glycerol method with the clear flour. The writer finds that with the Hertwig-Bailey method the glycerol flour mixture is preferably charred over a

<sup>1</sup> Presented by T. H. Hopper

<sup>2</sup> *Cereal Chem.*, 1924, 1: 82.

<sup>3</sup> *Ibid.*, 189.



free flame before placing in the muffle. If the crucible is placed directly into the muffle the mixture is likely to froth over the sides of the crucible. This additional handling required is an objectionable feature of this method.

TABLE 1\*.

*Results of ash determinations by the Hertwig-Bailey and proposed official A. O. A. C. methods.*

COLLABORATOR	SAMPLE MARK	HERTWIG-BAILEY METHOD		OFFICIAL A. O. A. C. METHOD	
		Ash	Time of ignition	Ash	Time of ignition
		<i>per cent</i>	<i>hours</i>	<i>per cent</i>	<i>hours</i>
1	A	0.380	..	0.416	..
		0.382	..	0.428	..
	B	1.240	..	1.301	..
		1.230	..	1.285	..
2	Patent	0.755	..	0.798	..
		0.758	..	0.806	..
		0.485	4½	0.482	5
		0.482	..	0.485	..
3	1	0.485	..	0.000	..
		0.295	2½	0.304	5½
	2	0.300	..	0.300	..
		0.430	..	0.432	..
4	1792	0.430	..	0.434	..
		0.43	..	0.47	6
		0.809	2	0.667	17
		0.752	4	..	..
5	1988	0.664	9	..	..
		0.673	2	0.611	17
		0.638	4	..	..
		0.594	9	..	..
6	1989	0.477	2	0.450	17
		0.481	4	..	..
		0.449	9	..	..
		0.574	2	0.442	17
7	2001	0.475	4	..	..
		0.428	9	..	..
		0.483	2	0.414	17
		0.457	4	..	..
8	2066	0.417	9	..	..
		0.512	2	0.457	17
		0.490	4	..	..
		0.470	9	..	..
9	2067	0.474	5	0.410	14
		0.597	5	0.570	14
		0.399	2-3	0.393	15
		0.415	2-3	0.400	15
10	B	0.465	2-3	0.453	15
		0.465	2-3	0.460	15
		1.288	1½	0.500	4½
		1.298	1½	0.504	4½
11	1	0.685	2	0.462	5
		0.771	2	0.468	5
		0.468	3	..	..
		0.474	3	..	..

\* From Report of C. H. Bailey, *Cereal Chemistry*, 1924, 1: 190.

Comments of collaborators on the glycerol method follow:

### COMMENTS.

A very large platinum dish was used, and no difficulty was experienced with frothing, though it took place. The sample with higher ash, apparently a first clear, required a much longer period for burning.

We can see no advantage gained by the glycerol-alcohol method and see danger of possible loss of particles, unless an extra large crucible is used.

*Hertwig-Bailey Method.*—Ignition—about 3 hours in muffle after preliminary charring over Bunsen, which required 2 hours with almost constant attention unless an exceptionally large platinum dish was used. Previous trials of this method have shown it to be accurate, but time consuming, owing to the attention required during the preliminary heating when the mixture froths.

The time saved by Method II averages a little less than an hour. Our opinion, however, is that this small amount of time saved in ashing does not justify bother of treating a large number of samples by Method II in routine work. Our results average a little higher by Method II.

TABLE 2.  
*Collaborative work on ash methods.*  
SAMPLE A

COLLABORATOR	METHOD I				METHOD II			
	(a)	(b)	Average	Time	(a)	(b)	Average	Time
C. E. Mangels	<i>per cent</i> 0.440	<i>per cent</i> 0.448	<i>per cent</i> 0.444	<i>hours</i> 16	<i>per cent</i> 0.446	<i>per cent</i> 0.442	<i>per cent</i> 0.444	<i>hours</i> 6
E. N. Frank Washburn Crosby Co. Minneapolis, Minn.	0.456	0.444	0.450	16	0.452	0.444	0.448	3½
C. H. Briggs Howard Laboratory Minneapolis, Minn.	0.430	0.435	0.433	16	0.426	0.444	0.435	16
G. S. Taylor University of Minnesota St. Paul, Minn.	0.444	0.434	0.439	5	0.440	0.432	0.436	5
H. G. Nelson International Milling Co. Minneapolis, Minn.	0.440	0.440	0.440	4½	0.452	0.448	0.450	4

SAMPLE B

C. E. Mangels	0.796	0.794	0.795	16	0.796	0.796	0.796	6
E. N. Frank	0.798	0.796	0.797	16	0.810	0.810	0.810	6½
C. H. Briggs	0.790	0.790	0.790	16	0.812	0.818	0.814	16
G. S. Taylor	0.796	0.796	0.796	5	0.816	0.818 0.800	0.811	5
H. G. Nelson	0.796	0.796	0.796	5	0.802	0.808	0.805	4

The frothing of the glycerol-alcohol mixture is an objectionable feature; while this difficulty is avoided by carefully charring over a free flame or use of a large dish, both procedures are open to criticism. Charring over a free flame requires the chemist's time and attention, and the use of a large ashing dish cuts down the capacity of the muffle furnace.

The comments of collaborators are unfavorable to the glycerol method; it may be worthy of further study before discarding, but the writer believes a more satisfactory short-time method can be devised.

#### USE OF CALCIUM ACETATE.

Some commercial laboratories now use the calcium acetate method for ash. The addition of calcium acetate prevents fusion of the ash and permits ignition at a higher temperature. The writer has made some studies on the use of calcium acetate, particularly with reference to the minimum quantity required to prevent fusion. The results of these studies are reported in Table 3. The procedure followed in making determinations is as follows:

A solution of calcium acetate is prepared so that 10 cc. contains the quantity of calcium oxide desired. Five gram samples of flour are weighed into tared crucibles, and 10 cc. of the calcium acetate solution is added. The flour is carefully mixed with the calcium acetate solution by means of a small stirring rod, and the adhering flour is removed from the stirring rod by a small piece of filter paper. The sample is dried

TABLE 3.  
*Effect of different quantities of calcium acetate on ash results.*  
SAMPLE A (5 GRAMS OF PATENT)

TEMPERATURE	CaO ADDED AS CALCIUM ACETATE	TIME	I	II	AVERAGE
°C.		hours	per cent	per cent	per cent
550	0	14	0.440	0.448	0.444
750	0.0209	3	0.514	0.516	0.515
750	0.0054	3	0.448	0.460	0.454
1000	0.0207	2	0.438	0.446	0.442
1000	0.0057	2	0.448	0.438	0.443

SAMPLE B (5 GRAMS OF FIRST CLEAR)

550	0	14	0.796	0.794	0.795
750	0.0209	3	0.878	0.884	0.881
750	0.0054	3	0.782	0.792	0.787
1000	0.0207	2	0.794	0.784	0.789
1000	0.0057	2	0.768	0.778	0.773

in an air oven and then ignited in a muffle. Ignition temperatures of 750°C. and 1000°C. were used. A blank was run on the calcium acetate solution by introducing 10 cc. into a tared crucible—evaporating and igniting at the same temperature.

All determinations were allowed to stay in the muffle longer than necessary to obtain a white ash, since it was desired to determine if over ignition would fuse the ash. The ash in all cases was almost white, and it contained much less gray than ash obtained by igniting at a low temperature.

Five milligrams of calcium oxide added as calcium acetate was sufficient to prevent fusion in both patent and clear flour when ignited 3 hours at 750°C. Five milligrams was sufficient to prevent fusion in patent flour when ignited 2 hours at 1000°C., but it did not prevent fusion of the first clear at 1000°C. The lower result indicates some loss by volatilization. Twenty milligrams of calcium oxide prevented fusion of both patent and clear at 1000°C. No explanation can be offered for the high results obtained with 20 mg. of calcium oxide at 750°C. These samples were returned to muffle for additional ignition, but the results were the same.

Wheat flour contains an acid ash and is easily fusible since the ash probably contains free phosphoric acid and acid salts of phosphoric acid. The addition of a base such as calcium oxide in the form of calcium acetate would tend to make the ash less fusible. The results show that patent flour requires less calcium acetate than clear flour. The use of a method permitting ignition at high temperature would considerably shorten the time required to obtain a carbon-free ash. Further study of the use of calcium acetate is desirable.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the following method used in collaborative work as the official method for ash and proposed by C. H. Bailey in 1922<sup>2</sup>, be adopted as official:

##### *Method I.*

Ignite a crucible; when cooled, weigh, and rapidly weigh into it 5 grams of flour. Ignite in a muffle at approximately 550°C., taking care that no portion of the muffle becomes sufficiently hot to fuse the ash. A light gray, fluffy ash should result. Cool the crucible and contents in a desiccator and weigh immediately after it reaches the temperature of the laboratory air.

As the methods now read, the official method for ash in flour is the same as for feeding stuffs, and no temperature of ignition is specified. The method recommended for final action is now in general use in flour laboratories, and collaborative work during two years has shown that it gives accurate results.

(2) That the referee study the glycerol, calcium acetate, and other methods, with a view to developing an optional method which requires a shorter time to obtain results than the method now generally employed, but which will give comparable results.

<sup>1</sup> For report of Sub-committee C. and action of the association, see *This Journal*, 1925, 8 278.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7 132.

## REPORT ON CHLORINE IN BLEACHED FLOUR.

By ARMIN SEIDENBERG (Chemical Laboratory, Department of Health, New York, N. Y.), *Associate Referee*.

At present there are two methods for the determination of chlorine in bleached flour that have been adopted as tentative by the association. In one method<sup>1</sup> the chlorine is extracted from the flour by a continuous extraction process. This method was considered cumbersome by O. S. Rask, who proposed another method<sup>2</sup>. In any case, a continuous extraction has been found generally unsatisfactory for many substances, due in part to the presence of sugar, which may form a thin layer over the fat and prevent its extraction. This type of method has been discarded by the association in the case of milk. The other tentative method proposed by Rask was also studied by Bailey<sup>3</sup> who modified it in some respects. However, the results secured by these methods were not in satisfactory agreement, and further study has been recommended.

It is the common belief of investigators in this field that the chlorine in bleached flour is to be found in or with the fatty extractable matter, the chlorine itself being readily soluble in the usual fat solvents.

## PROPOSED METHODS.

From a study of the various proposed methods it would seem that the discrepancies to be noted are due in a large measure to variations in the quantity of fat extracted and that the method that extracts the fat as completely as possible should give the most satisfactory results. It appears also that in almost all the results secured with unbleached flour the presence of slight amounts of chlorine have been noted. Since it is not normally present in fat, it can be assumed that this chlorine has been derived from the combined chlorine naturally present in flour, probably as an inorganic constituent, and that it has been carried along with the fat in the course of the extraction as an impurity. Of course, slight quantities of chlorine may be present due to unavoidable contamination while in a mill where chlorine bleaching is carried on. Nevertheless, in order that the present analytical procedure may be as effective as possible, it is important that the quantity of inorganic chlorine extracted be reduced to a minimum.

As it was not possible to bring the work on the determination of chlorine in bleached flour to a final conclusion, this report is confined to a comparative study of the various extraction methods in order to discover or devise a method that will yield the greatest amount of ex-

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1920, 189.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1922, 6: 68.

<sup>3</sup> *Ibid.*, 1923, 7: 130.

tractable matter under conditions that would indicate that it was as free as possible from inorganic chlorine.

Some suggestions of value bearing upon the procedure used by Rask and Bailey were made by the Referee on Cereal Products in a personal communication to the writer and incorporated in this method. As modified in accordance with these suggestions 20 grams of sample was treated with 150 cc. of petroleum ether, and after shaking at intervals during 1 hour the sample was allowed to rest overnight; the entire liquid portion was then filtered off into a large platinum dish and evaporated after the addition of 5 cc. of a 4 per cent alcoholic potassium hydroxide solution. After making the deductions secured from a blank for the reagents used (the slight change of weight due to the saponification of the fat being considered negligible), the extracted matter amounted to 1.02 per cent. The above procedure was repeated except that two additional extractions, for which 100 cc. of petroleum ether was used each time, were undertaken. In this instance the extracted matter amounted to 1.08 per cent or practically the same as with one extraction.

A neutral extraction method for lipoids proposed by Hertwig<sup>1</sup> seems to give quantitative results. In this method the material is first heated with 70 per cent alcohol, whereby it is brought to a condition that more readily permits the solubility of the fatty matter. On applying the method as originally outlined, using alcohol and ethyl ether, an emulsion resulted that filtered turbid even through a fine pore filter paper. It was very difficult and, in any case, inconvenient to clear the solution by centrifuging. On substituting petroleum ether for ethyl ether two liquid zones were formed, the upper of petroleum ether mainly and the lower consisting largely of alcohol. The petroleum ether was separated and evaporated, and additional extractions were made, but in all instances the extracted matter was less than that secured with the modification of the tentative method outlined above. There seemed no doubt, judging from these results, that some part of the fatty extract was held in the alcoholic layer.

Further investigation indicated that by the use of both ethyl and petroleum ether larger quantities of fatty matter could be extracted; however, emulsions were readily formed. Many trials were made, in which there were used different proportions of 70 per cent alcohol, 95 per cent alcohol, ethyl ether, petroleum ether, and water for washing, in order to develop a satisfactory procedure on the basis of some modification of the neutral extraction method proposed by Hertwig. The method that seemed to give the most satisfactory results and that yielded an extract equal to 1.25 per cent was essentially as follows:

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<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1923, 7: 91.

**METHOD.**

Weigh 10 grams of flour into a 500 cc. Erlenmeyer flask and add 30 cc. of 70 per cent alcohol. Warm the flask and contents very slightly until the flour is evenly distributed throughout the liquid. Then add 30 cc. of 95 per cent alcohol, stopper, and shake flask thoroughly for about 2 minutes. After the flask has completely cooled, add 50 cc. of ethyl ether to the contents and shake; then add 100 cc. of petroleum ether (B. P. 30°–75°C.) and shake again. Transfer the entire liquid contents to a separatory funnel. Add 25 cc. of petroleum ether to the flask, shake well, and add the liquid contents to the separatory funnel. Add 25 cc. of petroleum ether to the flask a second time, shake well, and pour the entire liquid contents into a separatory funnel. Add 30 cc. of water to the separatory funnel and shake the entire contents thoroughly. Allow the funnel to rest until the liquids, which should be entirely clear, have separated sharply into two layers, and then remove the lower layer. If necessary, make a second washing, using, however, only 10 cc. of water, and waiting until both layers are entirely clear before withdrawing the lower layer. (If a larger quantity of water is used, the lower layer becomes emulsified.) Drain the upper layer of solvents, containing the extractable matter into a beaker and evaporate slowly after the addition of 5 cc. of 4 per cent alcoholic potassium hydroxide.

**GLUTENIN IN FLOUR<sup>1</sup>.**

By PAUL F. SHARP (Agricultural Experiment Station, Bozeman, Mont.),  
*Associate Referee.*

The official method published in 1920 is as follows:

Deduct the sum of the potassium sulfate-soluble nitrogen, 11, and the alcohol-soluble nitrogen, 8, from the total nitrogen, 7, and multiply the difference by 5.7.

Because of the overlapping solubilities of the proteins of flour, this method gives too low results for glutenin.

It is not probable that a solvent for glutenin will be found that will not, at the same time, be a solvent for the other proteins of flour. According to present knowledge, the best method for determining glutenin would be to remove the other proteins with solvents in which glutenin is insoluble and then determine the protein in the residue. In removing the proteins other than glutenin from flour, two methods of attack are being tried: first, the use of two different solvents successively on the same sample of flour, such as a salt solution followed by alcohol and, second, the attempt to find one solvent that will remove all protein material except the glutenin. The first step will be to determine the quantity of protein removed by various solvents and then determine the protein composition of the material thus removed, by means of the nitrogen fractions after the manner of Bailey and Blish<sup>2</sup>. A point that will be stressed in the investigation will be the effect of ratio of solution to solvent on the quantity of protein removed.

<sup>1</sup> Presented by R. Hertwig.

<sup>2</sup> *J. Biol. Chem.*, 1915, 23: 345.

The points that have been investigated are as follows:

The quantity of protein removed from flour by extracting 4 grams with 100 cc. of alcohol of the following strengths: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 per cent by volume, was determined. This extraction was carried out by two methods—first, by shaking in a mechanical shaker for 1 hour and, second, by boiling under a reflux condenser for 1 hour. The latter method removed the most protein over the whole range of alcohol concentration.

Six grams of flour was extracted with 100 cc. of various salt solutions by shaking in a mechanical shaker for 1 hour. The following salts were used in both 5 and 10 per cent concentrations,  $K_2SO_4$ ,  $KCl$ ,  $MgSO_4$ ,  $MgCl_2$ , and  $CuSO_4$ , and in addition, 100 cc. of water. With the exception of  $MgCl_2$  the 5 per cent solution removed more protein than the 10 per cent solution.  $KCl$ ,  $MgSO_4$ , and  $CuSO_4$  removed more proteins than did the  $K_2SO_4$ . Nearly twice as much protein was removed with  $MgCl_2$  as with the other salts used. Water alone removed more protein than did any of the salts except  $MgCl_2$ . The residues from these various salt extractions were extracted with 150 cc. of 70 per cent alcohol by two different methods, first, shaking for 1 hour and, second, refluxing 1 hour. Refluxing removed more protein than did shaking. Slight effects of the various salts on the quantity of protein subsequently removed by alcohol were noted.  $CuSO_4$ , however, decreased the quantity removed to a considerable extent.

As these determinations were carried out on one flour only, definite conclusions can not be drawn until other flours are studied. The work is being continued.



REPORT ON SAMPLING OF FLOUR<sup>1</sup>.

By G. J. MORTON (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee*.

Provided the milling processes are properly controlled, the moisture content of freshly milled flour is undoubtedly uniform throughout. During storage, however, the moisture will increase, decrease, or remain stationary, depending upon atmospheric conditions, especially humidity. (See Graphs 3 and 4.) The rapidity of change is markedly influenced by rate of air circulation, provided, of course, the moisture in the flour and in the air has not reached a state of equilibrium.

Obviously, the sampling of flour of uniform composition is a simple operation. The increase of moisture by absorption or decrease by evaporation necessitates the development of a special method of sampling since the rapidity and extent of change are not uniform throughout the sack, the surface flour being affected in the greatest degree. Equalization of the moisture content by mixing thoroughly the entire contents of the sack would be correct but impracticable. Also the removal from the sack of a wedge-shaped sample representing proportionate quantities of flour of the different moisture contents would be equally impracticable although logical. Furthermore, a core of uniform diameter from top to bottom of the sack would contain disproportionately large quantities of flour from the central zone, which is of lesser volume than the outer. Therefore, based on experimental work already performed, a feasible method, one giving results approximating correctness, is to draw, by means of a suitable trier inserted with light pressure and slow rotation through the middle of the top of the sack in a horizontal position, a core of uniform diameter and from a depth approximating the distance from the edge to the center of the sack. This depth for 49 pound sacks, as determined by experiment, is approximately 6 inches. The depths obtained by calculation, but not by experiment, recommended for the other sizes of sacks are as follows:

<i>pounds</i>	<i>inches</i>
10	4
24½	5
98	7½
140	9

The sacks sampled in this manner should be selected from different locations in the pile, i. e., top, sides, and interior, the minimum number being as follows:

<sup>1</sup> Received too late for presentation or consideration at the meeting but printed as a part of the proceedings at the request of the Chairman of the Committee on Recommendations of Referees.

10 sacks from	100
15 sacks from	300
20 sacks from	600
30 sacks from	1,000

Place the composite sample immediately in air-tight containers without mixing, thus avoiding undue exposure. The analyst should prepare the sample by passing the flour through a domestic flour sifter three times, no further mixing being necessary. The trier devised for this purpose is made of heavy sheet copper and measures 12 inches long by 1 inch in diameter at the forward end and  $1\frac{3}{4}$  inches at the rear end, and bears marks indicating the different depths.

#### EXPERIMENTAL WORK.

The trier described was devised and adopted after numerous experiments with other types as, when properly used, i. e., inserted with a slow rotation, it cuts and delivers a core of uniform weight. Averages of 12 cores of each length, i. e., 3, 6, and 9 inches, showed quantities of flour delivered, respectively, as follows: 28.54, 56.17, and 85.00 grams; each 3 inch portion, whether from a 3, 6, or 9 inch core, weighed substantially the same, the slight differences being undoubtedly due to a variation in the density of the flour.

To approximate the degree of absorption and evaporation of moisture at different relative humidities, i. e., 10 per cent intervals from 30–90, inclusive, fourteen 4 pound samples, seven each of soft and of hard wheat flour (one sample for each R. H.) packed in air-tight cans immediately after milling were exposed for an average time of  $7\frac{1}{2}$  minutes by passing through a Boerner grain sampler three times and the final sample of 1 pound through a domestic hand flour sifter also three times, the sifting taking  $2\frac{1}{2}$  minutes. The soft wheat flour, although of a lower initial moisture content, lost more moisture at lower relative humidities than did the hard wheat flour, which contained initially a relatively high percentage of moisture. The data obtained seem to indicate that samples should be mixed at approximately 55 per cent R. H., as at higher or lower humidities marked gains or losses in moisture are likely to take place. The slight irregularity in results (see Graphs 1 and 2) may be accounted for by experimental errors and difficulty in maintaining uniform temperature and humidity conditions. The temperatures for the relative humidities from 30–90 per cent were, respectively, 80, 78, 81.5, 81, 81.5, 80, and 82°F.

To determine the proper method of mixing the sample by the analyst 1 pound of flour uniformly colored with charcoal and 1 pound uncolored were passed through a domestic flour sifter 1, 2, and 3 times, the third sifting producing a mixture that appeared upon very close examination

to be of uniform color. Rolling of a like sample on a large paper for  $\frac{1}{2}$  hour (150 times) failed to produce a mixture of even fair uniformity; consequently this method should never be used.

TABLE 1.  
*Zoning experiment.*

SACK NO.	PERIOD	INITIAL MOISTURE	MOISTURE IN ZONES AT ENDS OF PERIODS							
			Edges					Points of contact		
			1	2	3	4	5	1	2	3
	<i>days</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	13	13.84	11.71	12.40	12.98	13.08	...	...	...	....
3	20	13.94	11.21	11.79	12.18	12.65	12.99	....	....	...
5	27	13.89	11.00	11.44	12.00	12.24	12.57	12.81	12.95	13.06
7	34	14.02	11.44	11.80	11.96	12.16	12.30	12.70	12.91	12.95
9	41	14.12	11.15	11.55	11.76	12.12	12.34	12.65	12.73	12.57
11	48	13.95	11.31	11.49	11.74	11.92	12.26	12.41	12.47	12.60

TABLE 2.  
*Trier experiment.*

SACK NO.	PERIOD	INITIAL MOISTURE	MOISTURE AT ENDS OF PERIODS				
			ACTUAL*	Cores			
				3 in.	6 in.	9 in.	12 in.
	<i>days</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
2	13	14.07	12.61	12.28	12.57	12.92	12.79
4	20	13.85	12.27	11.23	12.16	12.52	12.55
6	27	13.88	12.14	...	11.95	12.31	12.28
8	34	13.82	12.22	....	11.87	12.25	12.52
10	41	13.99	12.03	....	11.93	11.89	12.42
12	48	13.99	11.93	....	11.69	11.83	11.91

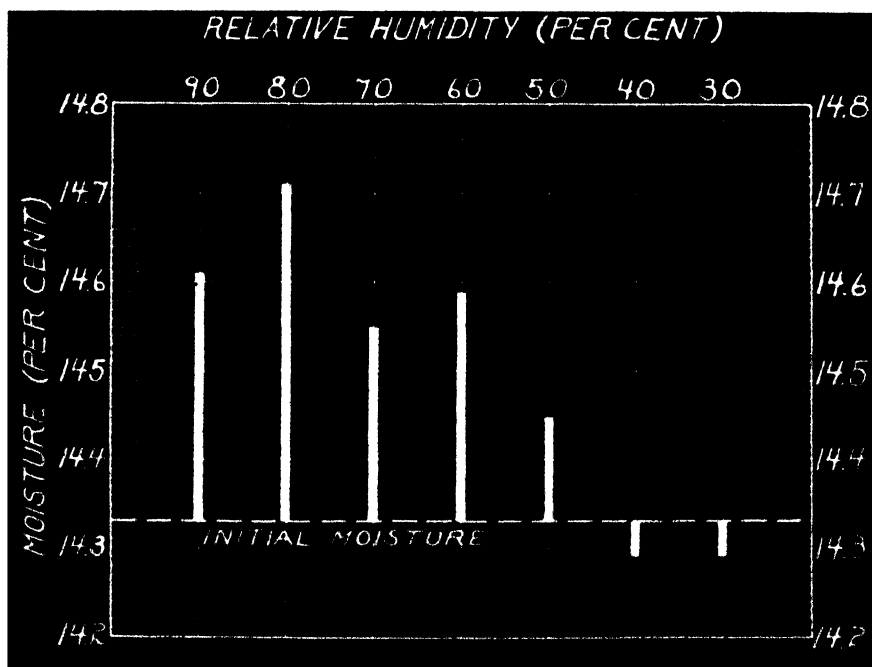
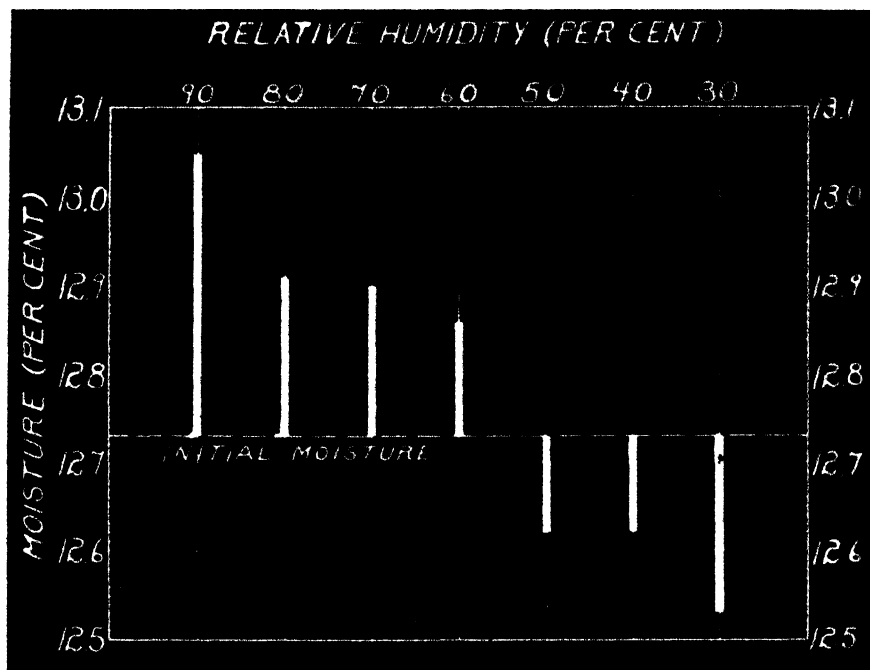
\* Entire contents of sack thoroughly mixed after cores were removed.

For the trier and the zoning experiments 12-49 pound sacks of hard wheat flour freshly milled were piled in a well ventilated room, two sacks to the tier, tiers being at right angles. One sack of each tier was used

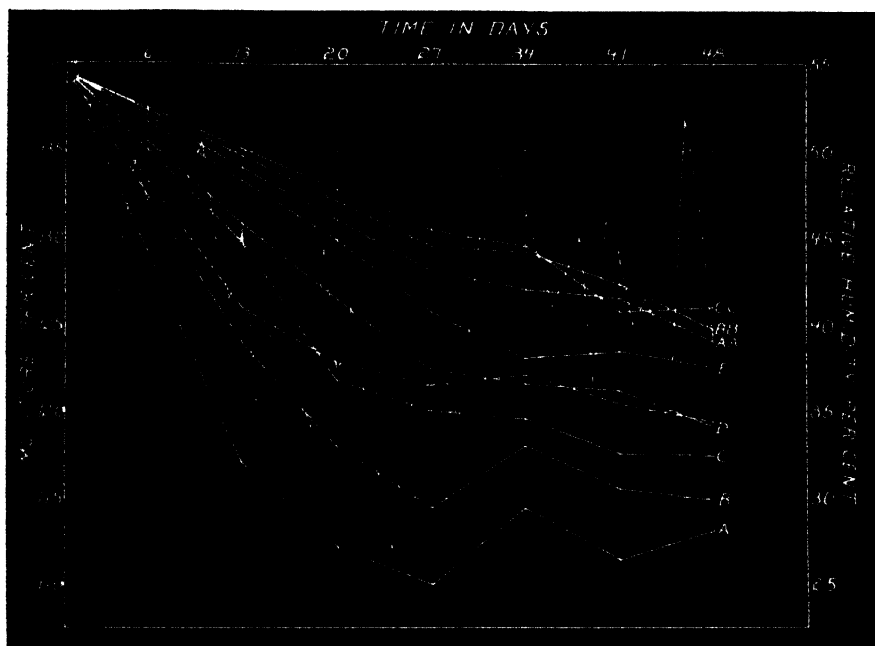
for determining the zoning or distribution of moisture and the other for experimenting with the different length triers, which were 3, 6, 9, and 12 inches. The zone samples were taken by means of the trier, an inch plug at a time from 12 places around the edges of the sack. These places, four on each side and two on each end, were equidistant. The zones were 1, 2, 3, 4, and 5, the fifth being as a matter of fact the inner portion of the fourth zone. Also samples, i. e., inch plugs, were drawn from zones 1, 2, and 3 at the two points of contact with the sacks above. Each zone sample was a composite of the plugs drawn from that zone, the edges and points of contact being considered separately. From the other sack, the companion one, the trier samples or cores were removed from the top at points  $1\frac{1}{2}$  inches apart and not closer than 3 inches to the edge of the sack. The four triers were inserted and removed simultaneously. The contents of the sack less the quantity of flour removed by the four triers were mixed thoroughly at approximately 55 per cent relative humidity by passing through a Boerner grain sampler five times, the resulting sample weighing approximately  $1\frac{1}{2}$  pounds. This sample, as were also the samples obtained from points of contact and the edges, was passed three times through a domestic flour sifter and placed in an air-tight container until analyzed. Moistures were determined in duplicate in vacuo at the temperature of boiling water, time of drying being 5 hours. The first series of samples, both zone and trier, were drawn on the thirteenth day and the others thereafter at intervals of seven days, the total time of experiment being 47 days. Daily temperature and humidity readings were taken.

The percentages of moisture, initial and at the six periods, both in the zones, odd numbered sacks, and in the different length cores, even numbered sacks, are set forth, respectively, in Tables 1 and 2. All these data are plotted on Graphs No. 3 and 4. The initial moisture as stated on the graphs, 13.94 per cent, is an average of the initial moisture percentages set forth in the two tables.

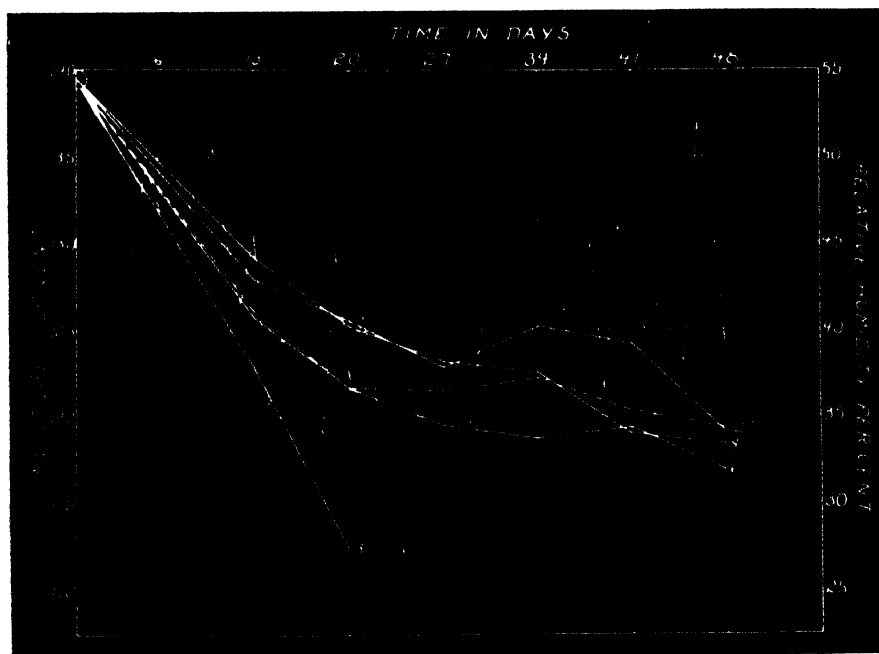
The actual losses in moisture, considering the sacks as a whole, were for the six periods 1.46, 1.58, 1.74, 1.60, 1.96, and 2.06 per cent. The losses in the first and second and, after the 20 day period, in the third zones were of course greater since the surface flour is more readily affected by changes in atmospheric conditions than the flour in the interior of the sacks. During the first period of the experiment the humidity increased from 30–50 per cent but decreased rapidly during the second period and after 24 days dropped to 27 per cent, increasing again during the fourth period to 49 per cent, and, during the fifth period decreased to 36.5 per cent with a rise to 46.00 per cent, while in the last period declining gradually to 38 with a sharp rise in three days to 52 and a fall to 39 per cent.



GRAPHS 1 AND 2.—EXPERIMENTS TO DETERMINE DEGREE OF ABSORPTION AND EVAPORATION OF MOISTURE AT DIFFERENT RELATIVE HUMIDITIES. GRAPH 1, SOFT WHEAT FLOUR. GRAPH 2, HARD WHEAT FLOUR.



GRAPH 3.—ZONING EXPERIMENT. THE BROKEN LINE INDICATES ACTUAL MOISTURE CONTENT OF THE FLOUR; A TO E THE MOISTURE IN ZONES 1 TO 5 FROM EDGES OF SACKS AND AA TO CC THE MOISTURE IN ZONES 1 TO 3 FROM POINTS OF CONTACT.



GRAPH 4.—TRIER EXPERIMENT. MOISTURE CONTENT OF THE FLOUR IN THE 3, 6, 9, AND 12 INCH CORE ACTUAL MOISTURE CONTENT OF FLOUR INDICATED BY THE BROKEN LINE.

Graph No. 4 shows that the six inch core approximates to actual moisture content most closely throughout the entire experiment, whereas the three inch core, being near the surface, is obviously of no value. On the other hand, the 9 and 12 inch cores representing greater portions of the interior flour gave high results. Eventually all lines would probably converge to one point provided atmospheric conditions remained constant.

Although no zoning and trier experiments were made to determine absorption of moisture, it is believed that the converse is true, that is to say, the absorption would be graduated from the outer surface to the interior portions substantially the same as is the evaporation.

The moisture determinations were made by Leonard Feldstein, Food and Drug Inspection Station, Denver, Colo.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That further experimental work be performed to determine whether the method of drawing samples described in this report gives substantially correct results on flour piled differently and held under other conditions of storage, and that if the method is found incorrect, a new method of sampling be devised.

(2) That further study be made of the rate of absorption and of evaporation of moisture when given quantities of flour are subjected to different though accurately controlled relative humidity and temperature conditions.

No report on the limit of accuracy in the determination of small quantities of alcohol was given by the referee.

No report on vinegars was given by the referee.

#### REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.), *Referee*.

The referee, being newly appointed in 1923, considered that his first duty should be to review the work of former referees on flavoring extracts. This was done, and the search of the literature showed that no action had been taken on eight recommendations of former referees. As a guide to the present and future work on the subject, these recommendations are set forth below:

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 277.

(1) 1916—That Howard's method and the present tentative method of determining the oil in cassia, cinnamon and clove extracts be further studied<sup>1</sup>.

(2) 1916—That the value of the test for the detection of vanilla resins be studied<sup>1</sup>.

(3) 1916, 17, 19, 20, 21—That a study of methods for the analysis of imitation vanilla preparations containing large quantities of coumarin and vanillin be undertaken<sup>1</sup>.

(4) 1916, 17, 19, 20, 21, 22—That the referee give consideration to the method adopted at the 1919 meeting of the association, as official, first action, for the determination of alcohol in orange and lemon extracts consisting only of alcohol, oil, and water, to the end that final action may be taken on the method at the 1923 meeting<sup>1</sup>.

(5) 1919—That the rapid methods for vanillin (Folin's quantitative), coumarin (Wichmann's qualitative), and lead number (Wichmann's quantitative), while meritorious, be held in abeyance until: (a) Sufficient data are collated on authentic samples of vanilla extracts to enable satisfactory interpretation of analyses; (b) the new lead number is submitted in some form in which it will not be confused with the present official method; and (c) a satisfactory quantitative rapid method for coumarin has been developed. Investigations along these lines by individuals, especially by the authors of the methods, are urged<sup>2</sup>.

(6) 1919, 20, 21—That the method suggested by Penniman and Randall for the determination of oil in lemon and orange extracts be studied in connection with the official method<sup>3</sup>.

(7) 1919, 20, 21, 22—That the referee give consideration to methods for the analysis of non-alcoholic flavors, as for example the determination of orange oil and lemon oil in mineral oil, cottonseed oil, etc.<sup>3</sup>

(8) 1921—That the official methods for the determination of citral in orange and lemon extracts and in orange and lemon oils be investigated<sup>3</sup>.

Before developing any new lines of investigation, the referee decided to begin clearing away as many as possible of these old recommendations. With this end in view, he selected for first consideration those with which he was already more or less familiar and thus in a position to judge the suitability of the methods covered by them. Of the eight recommendations listed, Nos. 4, 5, and 6 were selected for study.

The rapid colorimetric method devised by Folin and Denis<sup>4</sup> for the determination of vanillin is given below in a form suitable for inclusion in the *Methods of Analysis*, A. O. A. C.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1920, 3: 417-8; 533-4.

<sup>2</sup> *Ibid.*, 1921, 4: 479, 579.

<sup>3</sup> *Ibid.*, 1922, 6: 148.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1912, 4: 670.



## VANILLIN—METHOD II.

## REAGENTS.

(a) *Standard vanillin solution*.—Dissolve 0.1 gram of pure vanillin in water and dilute to 1 liter.

(b) *Phosphotungstic-phosphomolybdic acid*.—To 100 grams of pure sodium tungstate and 20 grams of phosphomolybdic acid (free from nitrates and ammonium salts), add 100 grams of sirupy phosphoric acid (containing 85 per cent  $H_3PO_4$ ) and 700 cc. of water. Boil over a free flame for 1½–2 hours; cool; filter, if necessary; and make up with water to 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

(c) *Sodium carbonate solution*.—Prepare 1 liter of a saturated solution of pure sodium carbonate.

(d) *Lead solution*.—Dissolve 50 grams each of basic and neutral lead acetate in water and make up to 1 liter.

## PROCEDURE.

Transfer to a 100 cc. volumetric flask such a quantity of the sample as will contain from 8–12 mg. of vanillin (usually 5 cc. will be the proper quantity). Add 75 cc. of tap water, at room temperature, and 4 cc. of the lead solution. Dilute to 100 cc. with water and mix. Filter through a dry filter paper and pipet 5 cc. of the clear filtrate into a 50 cc. volumetric flask. Into another 50 cc. volumetric flask pipet 5 cc. of the standard vanillin solution. To each of these flasks add from a pipet 5 cc. of the phosphotungstic-phosphomolybdic acid reagent, allowing the reagent to flow down the neck of the flask in such a way as to wash down the vanillin solution that may be on the sides of the flask. Mix the contents of the flasks by rotating and after 5 minutes dilute contents to 50 cc. with the sodium carbonate solution. Mix thoroughly by inverting the flasks several times and allow to stand for at least 10 minutes so that the precipitate that forms may separate completely. Filter the solutions through dry filter papers and compare the blue colors of the clear solutions in a colorimeter. Report result as grams of vanillin per 100 cc. of extract.

It was generally recognized, as a result of the work done by the association in 1919, that the above described method for vanillin is both short and accurate<sup>2</sup>. Leach<sup>3</sup> makes the following statement regarding it: "It yields accurate results, requires but 5 cc. of the material, and is exceedingly rapid". Twenty-one authentic samples of vanilla extracts were analyzed in the Water and Beverage Laboratory of the Bureau of Chemistry by this method and also by the official method<sup>4</sup>. The results obtained are given in Table 1<sup>5</sup>.

<sup>1</sup> For Method I, see *Assoc. Official Agr. Chemists, Methods*, 1925, 349.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1921, 4: 468.

<sup>3</sup> *Food Inspection and Analysis*, 4th ed., 1920.

<sup>4</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 349.

<sup>5</sup> Wilson and Sale. *Ind. Eng. Chem.*, 1924, 16: 301.

TABLE 1.  
*Vanillin in authentic vanilla extracts.*

OFFICIAL METHOD	FOLIN AND DENIS METHOD	OFFICIAL METHOD	FOLIN AND DENIS METHOD
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.16	0.14	0.14	0.15
0.15	0.14	0.24	0.23
0.14	0.11	0.11	0.12
0.14	0.12	0.13	0.14
0.08	0.08	0.14	0.12
0.18	0.17	0.12	0.11
0.09	0.11	0.13	0.11
0.23	0.22	0.09	0.07
0.19	0.22	0.15	0.14
0.22	0.18	0.13	0.12
0.22	0.20		

It will be noted from Table 1 that the agreement between the two methods is excellent. Adoption of the Folin and Denis method as official in 1919 was deferred because of the lack of a rapid quantitative method for the other determinations that are made together with that of vanillin, in the official method. In the Water and Beverage Laboratory, however, it has been found that the colorimetric method is very useful, and it is believed that it should be given a place in *Methods of Analysis*, A. O. A. C. It is also felt that the adoption of this method will give an impetus to the revision of the official method for coumarin.

The quantitative method for the determination of the lead number in vanilla extracts, as devised by Wichmann, is given below in a form suitable for inclusion in *Methods of Analysis*, A. O. A. C.

#### LEAD NUMBER—METHOD II.

##### REAGENTS.

(a) *Lead acetate solution*.—Dissolve 80 grams of neutral lead acetate in distilled water that has been recently boiled, dilute to 1 liter, and filter if the solution is not clear.

(b) *Dilute sulfuric acid*.—1 + 1.

(c) *Alcohol*.—95 per cent by volume.

(d) *Acetic acid*.—Glacial.

##### PROCEDURE.

Place 175 cc. of boiled distilled water in a round bottom flask of 1 liter capacity. Add by means of pipets 25 cc. of the lead acetate solution and 50 cc. of the sample. Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. The hole in the board should be of such size that when the flask contains 50 cc. of liquid, the level of the liquid will be even with the top of the board, or slightly above it. Connect the flask to a condenser, and with a moderate flame distil 200 cc. into a volumetric flask. Calculate the approximate alcohol content of the extract from the specific gravity of the distillate. (For accurate results, redistil over alkali.) Transfer the residual solution to a 100 cc. volumetric flask by means of

<sup>1</sup> *J. Ind. Eng. Chem.*, 1921, 13: 414. For Method I, see *Assoc. Official Agr. Chemists, Methods*, 1925, 350.

carbon dioxide-free water and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc. with carbon dioxide-free water, mix, and filter through a dry filter (Solution A).

Pipet 10 cc. of Solution A into a 250 cc. beaker and add 25 cc. of water, 2 cc. of the dilute sulfuric acid (1 + 1), and 100 cc. of 95 per cent alcohol. Stir, and allow to settle overnight. Filter on a Gooch crucible, wash with 95 per cent alcohol, dry, ignite at low redness, cool in a desiccator, and weigh.

For the blank determination, proceed as before, but use 5 drops of the glacial acetic acid in place of the sample and distil 150 cc. instead of 200 cc. The difference between the two weights of lead sulfate multiplied by 13.6646 gives the lead number of the extract. Report as "Lead number—Wichmann".

The above method was not published until 1921, but it had been tested by the association in 1919. Adoption of it was deferred until data on authentic samples could be obtained. Many such data are now available, and it has been noticed that a number of analysts are determining the lead number by this method, designating it as "Lead number—Wichmann", even though the method has not yet been published in books on food analysis. The lead number was determined in the Water and Beverage Laboratory by both this method and the official method on 27 samples of authentic vanilla extract. The results obtained are given in Table 2.

TABLE 2.  
*Lead number of authentic vanilla extracts*

OFFICIAL METHOD	WICHMANN METHOD	OFFICIAL METHOD	WICHMANN METHOD
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.12	0.18	0.04	0.23
0.08	0.18	0.10	0.19
0.04	0.07	0.03	0.06
0.07	0.12	0.51	0.70
0.04	0.02	0.59	0.72
0.65	0.86	0.21	0.32
0.78	1.05	0.19	0.25
0.18	0.29	0.01	0.09
0.13	0.13	0.11	0.24
		0.53	0.70
		0.77	0.83

It has been generally recognized that the Wichmann method gives higher results than the official method because the reaction goes to completion, whereas, in the official method the reaction is probably incomplete. The data in Table 2 indicate that such a conclusion is correct. It is not believed that the adoption of this method will result in any confusion, provided the analyst will indicate in his report which method he employs. Therefore, it will be recommended for adoption as an official method, first action. In the opinion of the referee, the present official method for lead number should be retained for 2 or 3 years until the

Wichmann method and data obtained by it become thoroughly familiar to analysts and then it should be dropped.

In connection with the method for lead number, a matter of minor importance was investigated. It has been proposed by Clemens<sup>1</sup> that the lead be determined by the chromate method instead of by the sulfate method. The chromate method is set forth below in a form suitable for inclusion in *Methods of Analysis, A. O. A. C.*:

*Determination of Lead as Lead Chromate.*

REAGENTS.

*Potassium dichromate solution, approximately N/10.*—Dissolve 5 grams of pure crystallized potassium dichromate ( $K_2Cr_2O_7$ ) in water and dilute to 1 liter.

PROCEDURE.

Pipet 10 cc. of the clear filtrate from the lead precipitate (Solution A, Method II) to a 400 cc. beaker and add 2 cc. of glacial acetic acid, 25 cc. of water, and 25 cc. of the potassium dichromate solution. Heat the beaker and contents immediately with a moderate flame and continue heating until the precipitate changes in color from yellow to orange. Then filter the solution through a weighed Gooch crucible provided with an asbestos mat and wash thoroughly with hot water and then with a few cc. each of alcohol and ether. Dry at 100°C., cool in a desiccator, and weigh. Determine the lead in the blank in the same manner. The difference in weights of lead chromate multiplied by 12.8217 is the lead number.

J. B. Wilson of the Water and Beverage Laboratory determined the lead number on 10 authentic samples of vanilla extracts both by the official sulfate method and the chromate method, and his results are contained in Table 3.

TABLE 3.  
*Lead number of authentic vanilla extracts.*

Official sulfate method	0.01	0.85	0.90	0.19	0.71	0.72	0.29	0.05	0.70	0.87
Chromate method	0.04	0.89	0.95	0.18	0.70	0.72	0.31	0.07	0.69	0.88

The chromate method is standard for the determination of lead, and its accuracy is unquestioned. Moreover, it is not necessary to allow the precipitate to stand overnight as in the case of the sulfate method and also there is a marked saving in alcohol reagent.

Penniman and Randall's gasoline-calcium chloride method<sup>2</sup> for the determination of essential oils in certain extracts was subjected to an investigation. One of the authors, W. W. Randall, kindly furnished a detailed description of the method that contained much useful information. However, as the essential directions for carrying out the method

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 79.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1914, 6: 926.

are given in the literature cited, a description will not be included here. A modification of the Penniman-Randall method, requiring 1 cc. of hydrochloric acid (1 + 1) and water instead of the acidulated solution of calcium chloride, was also tested. Synthetic extracts of lemon, orange, peppermint, anise, and nutmeg were manufactured in the Water and Beverage Laboratory and issued to the collaborators, W. W. Randall, J. B. Wilson, and the referee. The results obtained are given in Table 4.

TABLE 4.  
*Determination of oil in extracts of known composition.*

METHOD	LEMON			ORANGE			PEPPER- MINT	ANISE	NUTMEG
	1	2	3	1	2	3	2	2	2
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Official— by polarization	5.96	6.02	5.85	5.52	5.61	5.61			
Official— by precipitation	5.48	5.35	5.40	5.24	5.15	5.15	2.73	3.33	2.12
	5.45	5.30	5.45	5.30	5.15	5.20	2.63	3.33	2.12
			5.45			5.25	2.77	3.27	2.18
			5.55			5.10	2.71	3.22	2.11
								3.33	2.12
By gasoline— calcium chloride								3.33	2.17
								3.37	2.18
								3.32	2.11
	5.54	5.35	5.26	5.67	5.10	5.74	3.43	2.93	2.12
	5.63	5.23	5.49	5.69	5.49	5.62	3.74	3.07	2.16
		5.30			5.40		3.76	2.97	2.28
		5.49			5.29		3.82	3.02	2.21
		5.54			5.45		3.43	3.13	
		5.63			5.53		3.68	3.27	
							3.76	3.07	
							3.62	3.22	
							3.43		
							3.47		
							3.46		
							3.62		
By gasoline— acidulated water	5.54	5.29	5.35	5.50	5.25	5.39	3.03	3.03	1.92
	5.50	5.41	5.49	5.46	5.49	5.51	3.03	3.13	2.02
		5.48	5.44		5.54	5.56	2.97	3.07	1.88
		5.46	5.52		5.62	5.56	3.02	3.02	1.91
							3.03	2.93	1.92
							3.13	2.93	1.82
							3.17	2.87	1.78
							3.12	3.12	1.81
							2.93	2.93	2.02
							3.13	3.03	2.12
							2.97	2.87	1.98
							3.02	2.91	2.21
Oil present	5.5	5.5	5.5	5.5	5.5	5.5	3.26	3.27	2.25

The data in Table 4 are summarized in Table 5.

TABLE 5.  
*Summary of data in Table 4.*

METHOD		LEMON OIL	ORANGE OIL	PEPPER- MINT OIL	ANISE OIL	NUTMEG OIL
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Official— by polarization	Average	5.94	5.58			
	Maximum	6.02	5.61			
	Minimum	5.85	5.52			
Official— by precipitation	Average	5.43	5.19	2.71	3.31	2.15
	Maximum	5.48	5.30	2.77	3.37	2.18
	Minimum	5.30	5.10	2.63	3.22	2.11
Gasoline— calcium chloride	Average	5.45	5.50	3.60	3.08	2.19
	Maximum	5.63	5.74	3.82	3.27	2.28
	Minimum	5.23	5.10	3.43	2.93	2.12
Gasoline— acidulated water	Average	5.45	5.49	3.05	2.99	1.95
	Maximum	5.54	5.62	3.17	3.13	2.21
	Minimum	5.29	5.25	2.93	2.83	1.78
Present		5.5	5.5	3.26	3.27	3.25

The data in Tables 4 and 5, in the opinion of the referee, are not sufficiently conclusive to warrant the adoption of the new methods. The variation in individual results is greater by the new methods than by the official methods. Moreover, it is the opinion of the referee that much greater care is necessary in carrying out the new methods, which require the accurate measurement of small quantities of the highly volatile reagent, gasoline b. p. 40°–65°C.

The data in Tables 4 and 5, however, suggest a modification of the official precipitation method for the determination of oil in lemon and orange extract. In this method a solubility correction is made for lemon oil but not for orange oil. If it is assumed that this correction is made because of the solubility of the non-terpene constituents, then a similar though perhaps somewhat smaller correction should be made in the case of orange extracts. It is not believed that any material error will be introduced if the same corrections are made for orange extracts as for lemon extracts. Applying the correction of 0.4 per cent to the figures for orange oil obtained by the official method, as set forth in Table 5, there is obtained an average of 5.59 per cent, maximum 5.70, minimum 5.50, which figures agree quite well with the quantity actually present, which was 5.5 per cent.

At the 1919 meeting of the association, an alternative official method<sup>1</sup> was adopted, first action, for the determination of alcohol in orange and

<sup>1</sup> Hortvet and West. *J. Ind. Eng. Chem.*, 1909, 1: 84.

lemon extracts, consisting only of alcohol, oil, and water. In this method, the percentage by volume of alcohol is calculated by means of a formula, from the specific gravity of the extract, the percentage of oil in the extract, and the approximate specific gravity of the oil. The method has been published in *The Journal*<sup>1</sup> in a form suitable for inclusion in the *Methods of Analysis, A. O. A. C.*

The accuracy of the method was tested by the association in 1919<sup>2</sup>, and it was found that it gave very satisfactory results; in fact, the results were more satisfactory than those obtained by the official method. The association tested the method only on lemon and orange extracts, but the authors of the method determined alcohol in almond, cinnamon, clove, cassia, lemon, orange, peppermint, nutmeg, and wintergreen extracts by it and obtained good results. It has also been tested in the Water and Beverage Laboratory on 5 extracts of known composition, the figures for percentage of oil and specific gravity obtained by from one to three analysts being used. The results obtained are given in Table 6.

The data in Table 6 show that the method is accurate except with regard to orange extract when the oil is determined by the official precipitation method. If the figures for content of orange oil are corrected by the addition of 0.4 per cent, the new figures for alcohol become 85.2, 85.3, and 85.3, respectively, which are quite satisfactory, since the quantity of alcohol actually present was 85.1 per cent. Since the method in question is both rapid and accurate, it is proposed to recommend it for adoption as an alternative official method, second action.

The recommendations of the referee follow:

#### RECOMMENDATIONS<sup>3</sup>.

It is recommended—

(1) That the Folin and Denis rapid colorimetric method for the determination of vanillin in vanilla extract and its imitations, described in this report, be adopted as an alternative official method. (First action.)

(2) That the Wichmann method for the determination of the lead number of vanilla extract and its imitations, described in this report, be adopted as an alternative official method. (First action.)

(3) That the chromate method for the determination of lead, described in this report, be adopted as an alternative official method. (First action.)

(4) That the words "of lemon" be deleted from the sentence "If oil of lemon is present in amounts over 2 per cent \* \* \*" in the present official method for the determination of oil in lemon and orange extracts.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 308.

<sup>2</sup> *Ibid.*, 1921, 4: 472.

<sup>3</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 276.

TABLE 6.  
*Calculation of alcohol content by the formula of Hortvet and West\*.*

METHOD	LEMON EXTRACT			ORANGE EXTRACT			PEPPERMINT EXTRACT			ANISE EXTRACT			NUTMEG EXTRACT		
	Oil found	Alcohol by formula	Alcohol present	Oil found	Alcohol by formula	Alcohol present	Oil found	Alcohol by formula	Alcohol present	Oil found	Alcohol by formula	Alcohol present	Oil found	Alcohol by formula	Alcohol present
	per cent	per cent by volume	per cent by volume	per cent	per cent by volume	per cent by volume	per cent	per cent	per cent by volume	per cent	per cent	per cent by volume	per cent	per cent	per cent by volume
Official— by polari- zation	5.96	85.0	85.1	5.52	85.4	85.1									
	6.02	85.0	85.1	5.61	85.3	85.1									
	5.85	85.1	85.1	5.61	85.3	85.1									
Official— by precip- itation	5.47	85.4	85.1	5.27	85.6	85.1	2.71	78.9	78.4	3.31	78.5	78.4	2.15	79.5	79.2
	5.32	85.5	85.1	5.15	85.7	85.1									
	5.44	85.4	85.1	5.18	85.7	85.1									
Gasoline— calcium chloride	5.59	85.3	85.1	5.68	85.2	85.1	3.60	78.3	78.4	3.08	78.6	78.4	2.19	79.5	79.2
	5.42	85.5	85.1	5.38	85.5	85.1									
	5.38	85.5	85.1	5.68	85.2	85.1									
Gasoline— acidulated water	5.52	85.4	85.1	5.48	85.4	85.1	3.05	78.7	78.4	2.99	78.6	78.4	1.95	79.7	79.2
	5.41	85.5	85.1	5.48	85.4	85.1									
	5.45	85.4	85.1	5.50	85.4	85.1									

\* The approximate specific gravities of the oils are as follows: Lemon, 0.85; orange, 0.84; peppermint, 0.92; anise, 0.98; nutmeg, 0.89. The specific gravities of the extracts were found to be as follows: Lemon, 0.8293; orange, 0.8287; peppermint, 0.8580; anise, 0.8601; nutmeg, 0.8565.



(5) That the Hortvet and West method for the determination of alcohol in extracts consisting only of oil, alcohol, and water, as published in *This Journal*<sup>1</sup>, be adopted as an alternative official method. (Second action; first action was taken in 1919.)

(6) That the referee for next year continue clearing away the old unacted-upon recommendations listed in this report.

## REPORT ON MEATS AND MEAT PRODUCTS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.),  
*Referee.*

The work on analytical methods was divided, W. C. Powick, Associate Referee on Methods of Analysis working on methods for the determination of sugar in meats and the referee working on a method for the determination of nitrites in cured meats. The work on the separation of meat proteins has been continued by W. W. Ritchie, associate referee on this subject. No report of progress has been received from Ritchie up to the present date.

Powick's work is covered by his report as associate referee. The referee is in agreement with his conclusions, namely, that the present tentative method for sugar in meats is not dependable and that further studies leading either to the development of a new method or the detection and correction of the faults in the present tentative method is eminently desirable. Since the present tentative method is the most dependable known, it is recommended that it be retained in the *Book of Methods* pending the development of a more trustworthy method.

The method for the determination of nitrites in cured meats worked out in the referee's own laboratory was made necessary by developments in connection with certain experimental work. Details of this method are as follows:

### *Method for the Determination of Nitrites in Cured Meats.*

Weigh 5 grams of the finely comminuted and thoroughly mixed sample into a 50 cc. beaker. Add approximately 40 cc. of nitrite-free water heated to a temperature of 80°C. Mix thoroughly by stirring with a glass rod, taking care to break up all lumps, and transfer to a 500 cc. graduated flask. Wash out the beaker and rod thoroughly with successive portions of the hot water, adding all washings to the flask. Add sufficient hot water to bring the contents of the flask to a volume of approximately 300 cc., transfer the flask to the steam bath, and let stand for 2 hours with occasional shaking. Add 5 cc. of saturated mercuric chloride solution and mix. Cool to room temperature, make up to the mark with nitrite-free water, and mix again. Filter, and determine nitrite nitrogen in a suitable aliquot according to the method for nitrites in water<sup>2</sup>, reporting results as parts of sodium nitrite per million.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1922, 5: 308.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 85.

This method has been thoroughly tested by at least five analysts and has been found to give concurrent results in the hands of all. It is recommended for adoption as a tentative method<sup>1</sup>.

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No report on the separation of meat proteins was made by the associate referee.

## REPORT ON METHODS OF ANALYSIS FOR MEATS AND MEAT PRODUCTS.

By W. C. Powick (Bureau of Animal Industry, Washington, D. C.),  
*Associate Referee.*

The Associate Referee on Meats and Meat Products has directed his attention particularly to the tentative method for the determination of reducing sugars, the feature of which is the use of phosphotungstic acid for the removal of the non-coagulable proteins, creatinin, etc. This method has been used by the associate referee in the analysis of normal beef flesh, flesh from emaciated cattle, and various edible viscera of cattle, sheep, and swine, some of the samples being analyzed as soon as practicable after slaughter, and some after a week or more in cold storage.

In many cases the method appeared to be clean cut and yielded apparently satisfactory results. In other cases, however, trouble was experienced in the final reduction, the Fehling's solution-meat broth mixture remaining a clear blue until the last minute of boiling, when a rapid separation of yellow, more or less colloidal, cuprous oxide occurred, making it impossible to continue the determination. Such results appeared to bear no special relation to the character or age of the material being analyzed, although they were observed more frequently, if anything, in the old than in the fresh samples. In any case, the method in its present form appears to be untrustworthy.

A few experiments have been made to locate the source of the difficulty, but thus far without success. While the work is not complete, present indications tend to show that the trouble is not due to incomplete removal of creatinin, to the presence of 1 : 18 phosphotungstic acid in the specimens of 1 : 24 phosphotungstic acid employed, or to variations in the experimental conditions, length of standing, etc., obtained at different stages of the investigation. At the same time, definite conclusions to this effect would be premature.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 277.

The associate referee is impressed with the fact, however, that even though the difficulty mentioned should be removed, the method in question, in common with other reduction methods, will still be subject to the criticism that it determines the reducing capacity of a somewhat arbitrarily chosen fraction rather than that of the reducing sugars as such. Only in so far as the reducing non-sugars have been removed, will the results be valid for reducing sugars. But in the present state of knowledge of the chemistry of muscular tissue, no guarantee can be had that this has been accomplished.

The fermentation methods appear to be more specific for sugars than are the reduction methods; but until recently no really satisfactory fermentation method appears to have been proposed. An improved method that gives promise of overcoming the sources of error inherent in most other fermentation methods, however, has recently been reported by Costantino<sup>1</sup>, and the associate referee is of the opinion that the possibilities of this method should be investigated.

No report on gelatin was made by the referee. The recommendations approved by Sub-committee C have been published<sup>2</sup>.

## REPORT ON SPICES AND OTHER CONDIMENTS.

By R. E. ANDREW (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Referee*.

In the early part of 1924, the Referee on Spices and Other Condiments wrote to fifty spice trade chemists and spice dealers and importers asking for suggestions on the A. O. A. C. work for this year. A number of suggestions were received and considered, the most important of which related to the method of sampling.

G. Biston, of St. Louis, kindly brought this matter before the American Spice Trade Association, which was in convention in that city. The referee's letter requesting that association to consider this phase of spice analysis was read into the minutes of the meeting and, after open discussion, was referred to a committee. This is a very important phase of spice analysis, especially from the view-point of the trade chemist and is a matter that should be settled to the satisfaction of both the trade and control chemists.

## COLLABORATIVE WORK.

The collaborative work this year has been a continuation of the study of the methods for the analysis of salad dressings. Because referees

<sup>1</sup> *Arch. ital. biol.*, 1924, 72: 222.

<sup>2</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 277.

have had difficulty in manufacturing a dressing of known composition that would not separate by the time it reached the collaborators, it was decided this year to work on a single commercial brand. The E. R. Durkee Co. of Long Island City, N. Y., kindly sent the referee a supply of their 3 ounce size salad dressing, and this was used as the cooperative sample; in all cases it reached the collaborators in good condition.

The referee last year recommended methods<sup>1</sup> for the analysis of salad dressings. It has been suggested that these preparations be classified with egg products and, with this thought in mind, the referee has proposed procedures for the determinations of fat and lipid phosphoric acid that are substantially like those already tentative for egg products.

Samples were sent, and reports were received from the following: Otto F. Meyer, The H. J. Heinz Co., Pittsburgh; Marie L. Offutt, Food and Drug Inspection Station, New York City; Julius Hortvet, Dairy and Food Commission, St. Paul (work done by Otto C. Kueffner); Paul Rudnick, Armour and Co., Chicago (work done by Victor Conquest); and J. I. Palmore, Food Control Laboratory, Washington, D. C.

#### INSTRUCTIONS TO COLLABORATORS.

The methods given in the instructions sent to each collaborator for preparation of sample, total solids, lipid phosphoric acid, and fat have been published<sup>2</sup>. The following additional proposed methods for fat and lipid  $P_2O_5$  were also included:

##### *Fat—Proposed Method.*

Treat 5 grams of the sample in a loosely stoppered 200 cc. Erlenmeyer flask with a mixture of 10 cc. of alcohol (95 per cent), and 2 cc. of strong ammonium hydroxide, keeping the contents of the flask at the boiling point for 2 minutes, preferably on the steam bath. After cooling, extract the contents of the flask with three successive 25 cc. portions of ethyl ether, mixing and tamping the material thoroughly each time with a glass rod flattened at the end and pouring the extracts off by decantation into a 250 cc. beaker. Drain off the last 25 cc. portion as completely as possible, add another 12 cc. portion of the same ammoniacal alcoholic solution to the flask, and disintegrate the matted material as thoroughly as possible by means of the flattened glass rod, which may be left in the flask for this purpose. Return the flask to the steam bath and repeat the entire procedure, pouring the second set of ether extracts into the beaker containing the first set. (The second treatment with the ammoniacal alcohol mixture should be more gradual and somewhat longer than the first, so that the ether remaining in the flask may be evaporated off and the ammoniacal alcohol brought to the required boiling point without results disastrous to the determination, spattering.) Evaporate the combined extracts to *dryness* on the steam bath and extract the fat from the residue left in the beaker with successive portions (5 or 6 treatments, using about 15 cc. each time) of a mixture of equal volumes of ethyl ether and petroleum ether. Decant through a small filter paper into a weighed platinum dish and evaporate to dryness. Dry the residue in a water-jacketed oven at the temperature of boiling water to constant weight, weighing at hourly intervals.

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1925, 8: 176.

<sup>2</sup> *Ibid.*, 1924, 8: 172; *Assoc. Official Agr. Chemists, Methods*, 1925, 321.

*Lipoid P<sub>2</sub>O<sub>5</sub>—Proposed Method.*

Add 5 cc. of an alcoholic solution of potassium hydroxide (8 grams KOH to 100 cc. alcohol) for each gram of fat, as obtained in the preceding proposed method, evaporate to dryness, and char over asbestos. Treat the charred mass with dilute nitric acid (1 + 2), filter, and wash with hot water. Return the residue with the paper to the platinum dish and burn to a white ash. Treat again with dilute nitric acid, filter, and wash, uniting the filtrates. Determine the phosphoric acid by the official method.

## DISCUSSION.

The referee is of the opinion that salad dressing, since it is generally examined for the presence of eggs, could more properly be included in the chapter "Eggs and Egg Products" than under "Spices". If it is decided to make this change, then the tentative methods under egg products should be used, where possible, in the analysis of salad dressing. The work this year, therefore, has been confined to a study of the tentative methods proposed by the referee last year for salad dressing in comparison with certain of the tentative methods now in force under egg products.

The results as received from the collaborators in respect to the fat determination agree very well, but the lipoid phosphorus figures are rather surprising. It is the consensus of opinion that the Juckenack method<sup>1</sup> is not dependable in that it gives incomplete extraction, but from a study of the results shown in the table it will be seen that the tentative (Juckenack) method in most cases gives the higher results. It is quite

TABLE 1.  
*Summary of results on collaborative samples.*

COLLABORATOR	TOTAL SOLIDS	FAT		LIPOID P <sub>2</sub> O <sub>5</sub>	
		TENTATIVE METHOD	PROPOSED METHOD	TENTATIVE METHOD	PROPOSED METHOD
Otto F. Meyer	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	61.90	35.92	35.94	0.078	0.054
	62.16	36.09	36.03	0.074	0.051
Marie L. Offutt	61.71	37.22	37.04	0.071	0.066
	61.71	37.01	37.18	0.074	0.058
Otto C. Kueffner	61.53	37.08	37.49	0.067	. .
Victor Conquest	63.32	37.79	37.90	0.038	0.051
	63.39	37.81	37.89	0.044	0.048
J. I. Palmore	61.39	36.50	36.77	0.075	0.043
R. E. Andrew	61.65	36.68	36.74	0.053	0.067
	61.70	36.77	36.79	0.054	0.068

<sup>1</sup> *J. Assoc. Official Agr. Chemists*, 1924, 7: 408.

evident that this method should be studied again next year. Pending such further study no change in classification of this product is recommended this year.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended that the proposed methods for fat and lipid phosphoric acid, as herein described, be further studied in comparison with the methods for these determinations already tentative for egg products<sup>2</sup>.

#### REPORT ON CACAO PRODUCTS.

By E. M. BAILEY (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Referee*.

Experience has shown that the chief form of sophistication in cacao products consists in the addition of cacao shells or the retention of excess of shells, the addition of cocoa dust, the incorporation of foreign fat or the substitution of such fat for cacao fat, and, in case of those products made with milk, a deficiency of milk solids. The work of the several referees on cacao products during the last few years has been directed toward the perfection of methods for detecting these forms of adulteration. Commercial products or samples especially prepared in the laboratory have been the materials studied.

When the necessity of having investigational material of known history, preferably prepared so far as possible under factory conditions, became apparent, a plan was devised in the Bureau of Chemistry, Department of Agriculture, for the preparation of a considerable quantity of cacao products according to known formulas and under conditions obtaining in factory practice. This work was begun more than a year ago as indicated in the report of one of the referees at the last meeting<sup>3</sup>. The work was carried out under the supervision of representatives of the Bureau with the collaboration of a leading manufacturer, in whose plant the several products were prepared. This material has furnished the basis for the investigations of the past year.

#### OUTLINE OF WORK PROPOSED BY ASSOCIATE REFEREES.

V. A. Pease, the Associate Referee on Microscopical Methods, has devoted further study to the question of shell determination. This work has been carried on according to a definite program, and considerable progress has been made, but the point where collaborative work may profitably be requested has not yet been reached. A number of funda-

<sup>1</sup> For report of Sub-committee B and action by the association, see *This Journal*, 1925, 8: 264.

<sup>2</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 232.

<sup>3</sup> *J. Assoc. Official Agr. Chemists*, 1924, 8: 176.

mental points have not been established to the satisfaction of the associate referee, and she will, therefore, not make a separate report this year.

E. R. Miller, Associate Referee on Crude Fiber in Cacao Products, has continued his study of a modified procedure for fiber, and it was planned to study also the effect, if any, of the alkali treatment upon the fiber content of cacao products made by the Dutch process. However, so much of his time has been required in the examination of the raw materials entering into the experimental chocolate samples that the full referee program could not be accomplished.

Considerable time has been spent in the last two or three years in perfecting methods for the examination of cacao butter, and two tentative methods for this purpose have been adopted by this association. There seems to be no urgent need of further work in this particular direction as there is little, if any, evidence of adulteration in cacao butter as a separate commodity. W. F. Baughman, as associate referee on this subject, has proposed to study methods for the examination of milk chocolate with reference to the detection of foreign fats in the presence of both cacao and milk fats as a matter of more practical importance, and has prepared a report on this phase of the work.

#### COLLABORATIVE WORK ON AUTHENTIC CACAO PRODUCTS.

In addition to this program, it was thought to be distinctly worth while, in view of the authentic material available, to study collaboratively the official and tentative chemical methods for the examination of cacao products, as they are approved at this time, in order to discover their points of weakness and suggest the direction in which further effort should be made. Ready response was received to calls for collaboration and, at this time, reports from ten collaborating chemists have been received. No small amount of work was involved although the program was limited to those determinations of chief diagnostic importance. Because of the time required and the pressure of other work a number of reports were necessarily delayed. This, combined with the fact that this meeting is on an earlier date than usual, has prevented a sufficient study of results to be made, and a compilation of the figures obtained will, therefore, not be presented this year. The data afford the basis for many valuable comparative studies.

Acknowledgment is made directly to the analysts who engaged in the work, but the courtesy and cooperation of the chiefs of the several stations is also acknowledged and appreciated. The collaborating chemists were:

D. H. McIntire, Seattle, Wash.; J. Callaway, Jr., Savannah, Ga.; S. C. Rowe, Philadelphia, Pa.; M. L. Offutt, New York; C. A. Greenleaf, Cincinnati, Ohio; John T. Field, Minneapolis, Minn.; R. L. Horst, New Orleans, La.; Ferris and Stoner, Buffalo, N. Y.; and C. E. Goodrich, Baltimore, Md.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the study of methods for the estimation of shell in cacao products be continued.

(2) That methods for the detection of foreign fats in cacao products containing milk be continued.

(3) That the crude fiber content of alkali-treated cacao products be studied.

(4) That the methods for the determination of fat in cacao products proposed by Lepper and Waterman and by Feldstein be studied collaboratively.

No report on microscopical methods for cacao products was given by the associate referee.

No report on methods for the determination of crude fiber in cacao products was given by the associate referee.

No report on cacao butter was given by the associate referee, but the following paper was submitted:

DETECTION OF COCONUT AND PALM KERNEL OILS IN  
CACAO BUTTER AND FAT FROM MILK CHOCOLATE.

By WALTER F. BAUGHMAN (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee on Cacao Butter.*

The work this year consisted of determining the value of a qualitative test for coconut and palm kernel oils in cacao butter and the fat from milk chocolate. The following outline of the method was sent to the collaborators:

*Coconut and Palm Kernel Oils in Cacao Butter and Fat from Milk Chocolate*<sup>2</sup>.

## REAGENTS.

- (a) *Alcoholic potash*.—25 grams of potassium hydroxide in 200 cc. of ethyl alcohol.  
(b) *Saturated salt solution (use common salt)*.

## DETERMINATION.

Saponify 5 grams of the sample with 10 cc. of the alcoholic potash solution. Evaporate the alcohol on the water bath. Add 5 cc. of water and evaporate to remove last trace of alcohol. Dissolve the soap in 100 cc. of water; cool to room temperature; and add,

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1925, 8: 279.

<sup>2</sup> *Chem. Ztg.*, 1907, 31: 855; *Z. offent. Chem.*, 1908, 14: 67.



while stirring, 100 cc. of the saturated salt solution. Allow to stand 15 minutes and during this period stir several times. Remove the separated soap by filtration, using a Büchner funnel. To 100 cc. of the filtrate add, while stirring, 100 cc. of the saturated salt solution and allow to stand 15 minutes. Only a slight precipitate should appear. Filter, and slightly acidify the filtrate with hydrochloric acid. Run a blank on a sample of pure cacao butter at the same time. If the sample consists of pure cacao butter or fat from milk chocolate, the solution will remain clear when acidified. If coconut or palm kernel oil is present, the solution will become turbid or milky.

The collaborators were requested to apply this test to the following samples, which were sent to them:

## COMPOSITION OF SAMPLES.

NO.	CACAO BUTTER	MILK FAT	COCONUT OIL	PALM KERNEL OIL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	72	18	10	
2	68	17	15	
3	80	20		
4	72	18		10
5	68	17		15
6	100			

The collaborators subjected these samples to the test and reported the results given in the table. All six analysts reported positive results for Samples 1, 2, 4, and 5, which consisted of cacao butter, milk fat,

*Results of collaborative work.*

ANALYST	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
H. C. Waterman Bureau of Chemistry Washington, D. C.	positive	positive	very faintly positive	positive	positive	negative
J. K. Morton Bureau of Chemistry Washington, D. C.	"	"	"	"	"	"
P. L. Gowen Bureau of Chemistry Washington, D. C.	"	"	"	"	"	"
R. M. Hann Bureau of Chemistry Washington, D. C.	"	"	"	"	"	"
L. Katz U. S. Food and Drug Inspection Station New York City	"	"	positive			
Sumner C. Rowe U. S. Food and Drug Inspection Station Philadelphia, Pa.	"	"	faintly positive	"	"	"

and either coconut oil or palm kernel oil in various proportions. For Sample 3, which consisted of 80 per cent cacao butter and 20 per cent milk fat, four analysts reported "very faintly positive" results, one a "faintly positive" result, and the other a "positive" result. All six collaborators reported negative results for Sample 6, which consisted of pure cacao butter, and agreed that the intensity of the turbidity of the acidified solutions increased in the following order: 3, 4, 5, 1, 2. In other words, while a faint turbidity was obtained with Sample 3, which consisted of fat from milk chocolate, this turbidity was fainter than that obtained with the samples that contained coconut oil or palm kernel oil. The study of these results suggests the possibility that if the description of the method is changed so that the analyst will disregard the faint turbidity produced by the fat from milk chocolate the test will be satisfactory for the detection of coconut oil or palm kernel oil in cacao butter or the fat from milk chocolate.

It is recommended that further study be given to this test.

#### A METHOD FOR THE RAPID AND ACCURATE DETERMINATION OF FAT IN CACAO PRODUCTS.

By H. A. LEPPER and H. C. WATERMAN (Food Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture).

Anhydrous ethyl ether as a quantitative solvent for the fat of cacao products has three disadvantages. First, the weighed samples must be dried over sulfuric acid during a period of from 24–48 hours before extraction. Second, the general statement that "ether extract" is not pure fat can be shown to apply with particular force to cacao products. Leffmann and Beam<sup>1</sup> call attention to the solubility of theobromine—somewhat more than 1 part in 2,000—in cold ethyl ether, and make the following statement: "The extraction of the fat should be performed by means of petroleum spirit". Allen<sup>2</sup> finds theobromine soluble, 1 part in 3,125 parts of *anhydrous* ethyl ether, but insoluble in "petroleum spirit", and gives "petroleum ether" as a suitable fat solvent. But neither authority makes any statement concerning the quantity of theobromine actually extracted under the conditions of an ether-fat determination. The experiments of the writers, however, indicate that the quantity of non-fatty substance extracted is analytically significant. After thoroughly exhausting a cacao powder with petroleum benzine, 0.6–0.7 per cent of a substance consisting almost entirely of a nearly white powder of high melting point was extracted from it with anhydrous ether, under the conditions of the present official method<sup>3</sup>. This was accompanied

<sup>1</sup> Food Analysis, 2nd ed., 1905, pp. 273, 280.

<sup>2</sup> Commercial Organic Analysis, 4th ed., 1912, 6: 591.

<sup>3</sup> Assoc. Official Agr. Chemists, Methods, 1925, 345.

by a trace of a dark brown, resin-like material. Also, determinations of cacao and milk chocolate fat by means of petroleum benzine, while for the most part rather closely concordant, are from 0.3–0.6 per cent lower than those made by the official method (Tables 1 and 2). Third, it is well known to experienced food analysts that difficulty often arises in continuous extraction methods from the tendency of cacao products to run through, and if this be prevented by a dense filtering medium, filtration may be almost stopped before the extraction is completed.

To avoid some of the disadvantages of the use of anhydrous ethyl ether and continuous extraction methods, the writers have worked out a simple and rapid procedure employing petroleum benzine as the solvent. The figures obtained on various cacao products (Table 1) agree closely enough for a determination of this character and magnitude, and although the actual manipulation time is about the same as in the present official method, the *total* time, from the weighing of the samples to the report of the completed determinations, is very much less (Table 3).

The "fat" thus extracted does not, of course, consist exclusively of saponifiable glyceryl esters. Phytosterols and lecithins are soluble in petroleum benzine, and traces of resinous substance are not positively excluded. But the extraction of the last named impurity must at all events be less with petroleum benzine than with ethyl ether, as the experiments already mentioned have shown, and phytosterols and lecithins may perhaps reasonably be regarded as normal fat constituents.

#### PRELIMINARY EXPERIMENTS.

The first determinations were made by a modification of the official indirect method for fat in butter<sup>1</sup>. The samples were weighed in the crucibles; the fat was washed out with petroleum benzine; and the dried, fat-free residues were weighed. The fat solution showed an annoying tendency to "creep", however, and efficient stirring and grinding could not be performed in the Gooch crucible without danger of actual spilling. An asbestos mat thick enough to prevent running through did not leave sufficient room in the crucible for convenient manipulation. Also, the dried, fat-free samples absorbed moisture so rapidly that accurate weighing could only be done in closed weighing bottles. The determination by difference was therefore abandoned, Knorr tubes were substituted for the Gooch crucibles, and the fat was collected in weighed flasks.

#### PROPOSED METHOD.

Prepare in a Knorr extraction tube a tightly packed mat of asbestos purified as for the determination of crude fiber and carefully freed from coarse pieces. Wash the filter with alcohol, ether, and a little petroleum benzine. (*All petroleum benzine used in this determination must be redistilled below 60°C.*) Prepare the sample according to

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 276.

the official method<sup>1</sup> for cacao products and weigh 2-3 grams into the tube. Insert the tube into a rubber stopper in a filtering bell-jar connected to the suction through a two-way stopcock, taking care that no rubber particles adhere to the tip of the stem. Place a weighed 150 cc. Erlenmeyer flask at such a height that the tube stem passes through the neck into the flask. The stem of the tube should be lengthened if necessary. Fill the tube to about two-thirds of its capacity with the redistilled petroleum benzine, and by means of a rod having a flattened end stir the sample thoroughly, taking care to crush all lumps. Let stand 1 minute, and drain by suction. Do not use a suction so strong as to boil violently the collected solvent in the flask. Add the solvent from a wash-bottle, at the same time turning the tube between thumb and finger so that the sides of the tube are washed down by each addition. Repeat the extraction, with stirring, until the fat is removed. (Ten extractions will usually be sufficient.) Remove the tube with stopper from the bell, wash the traces of fat from the end of the stem with petroleum benzine, evaporate the solvent, and dry to constant weight at 100°C.

The fat-free sample may be used for the crude fiber determination.

#### ACCURACY OF THE PROPOSED METHOD.

To establish the accuracy of the results obtained, confirmatory evidence was necessary on two points: (a) That the difference between the figures given by the proposed method and those by the present official method do not represent fat—the official method giving in almost every case a significantly higher figure; and (b) that the fat extracted by petroleum benzine does not contain sugars or the cacao alkaloids.

(a) Samples that had been extracted by the proposed method were treated with anhydrous (held over sodium) ethyl ether, in accordance with the directions of the present official method<sup>2</sup>. No fat was obtained, but on evaporating the ether the white powder already referred to was deposited, with its accompanying trace of resinous substance in quantity sufficient to account within normal analytical limits for the observed differences between the results given by the two methods.

(b) No nitrogen (Kjeldahl) was dissolved by warm, dilute hydrochloric acid from 5 grams of fat extracted from a cocoa powder by redistilled petroleum benzine. No carbohydrate could be detected (Molisch test) in the hot water extract of 5 grams of fat from a sweet chocolate. Two grams each of sucrose, lactose, and dextrose were mixed and treated with about 50 cc. of redistilled petroleum benzine. The mixture was allowed to stand, with occasional shaking, for 48 hours. It was then filtered, and the filtrate was evaporated to dryness. No visible residue appeared, and a Molisch test was negative.

It should perhaps be added that the non-fatty solids must be finely divided, or readily reducible under the conditions of the analysis to such a state, if accurate results are to be obtained. This is of course true of commercial cacao preparations except nibs and "cocoa shells."

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 343.

<sup>2</sup> *Ibid.*, 7.

## PROBABLE IDENTITY OF THE NON-FATTY ETHER EXTRACT.

The statements of Leffmann and Beam and of Allen made it seem practically certain that the predominating constituent of this substance was theobromine. An anhydrous ether extract was prepared, however, from the residue of about 50 grams of a cocoa that had previously been carefully exhausted with redistilled petroleum benzine. From the dry residue of this extract cold ether removed a few milligrams of the dark brown, sticky, resin-like substance, leaving a white powder, about 0.3 gram. This gave a strongly positive murexide reaction, did not melt, but began to sublime at 285°–286°C. It was difficultly soluble in cold alcohol, and only slightly more so in hot alcohol. It separated slowly from saturated alcoholic solution in microscopic granules of no definite crystalline habit. The nitrogen content (1 sample only, but little more than 30 milligrams, was available) was estimated as 27.4 per cent by the Kjeldahl method. Pure theobromine is stated to sublime without melting at from 290°–295°C.; it contains 32.5 per cent of nitrogen.

The cursory examination thus indicated that the substance was impure theobromine. It was in any case clearly evident that it was not fat, and the expenditure of time and material necessary to secure a preparation sufficient for complete identification was not considered justifiable.

## TIME REQUIREMENT: PROPOSED VS. OFFICIAL METHOD.

A comparative summary of both the manipulation time and the delays involved in the proposed method and in the present official method will be found in Table 3. It will be seen that in the proposed procedure four samples require scarcely more time than does one. Four sets only of the apparatus used were available for these experiments. Even with this limited equipment, however, it was easily possible to weigh, extract, and report eight samples in one working day. It would doubtless be possible, with proper equipment and working conditions, to increase this number materially.

## SUMMARY.

The accuracy of petroleum benzine as a fat solvent for cacao products has been established, and a convenient manipulation for the rapid determination of the fat in cacao products with this solvent has been devised. It has been shown to possess advantages in rapidity, convenience, and accuracy over the present official method for cacao products.

TABLE 1.

*Determinations of the fat of typical cacao products by the proposed method and the present A. O. A. C. method.*

MATERIAL	PROPOSED METHOD	OFFICIAL METHOD
	<i>per cent</i>	<i>per cent</i>
Cocoa Powder.....	19.78	20.17
	19.64	20.27
	19.59	
	19.73	
	19.68	
Cocoa Powder.....	24.06	24.27
	23.90	
Cocoa Powder.....	26.35	26.25
	26.50	26.27
Cocoa Powder (alkali treated).....	29.87	30.30
	29.81	30.24
Bitter Liquor.....	51.26	51.56
	51.38	
Bitter Liquor.....	53.33	53.63
	53.30	53.66
Milk Chocolate .....	30.18	30.67
	30.37	30.80
Milk Chocolate.....	39.90	40.21
	39.91	40.25
Sweet Chocolate .....	36.40	36.75
	36.46	36.85

TABLE 2.

*Non-fatty ether extract from a cacao powder.*

PETROLEUM BENZINE		TOTAL ETHER EXTRACT OFFICIAL METHOD
Extracted by proposed method	Subsequently extracted by anhydrous ether	
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
19.59	0.72	
		20.27
19.73	0.67	
		20.17
19.68	0.57	
19.78	0.71	
Average 19.70	0.67	20.22

TABLE 3.  
*Manipulation time and delays, official and proposed methods.*

OFFICIAL METHOD		PROPOSED METHOD	
<i>4 samples</i>		<i>1 sample</i>	<i>4 samples</i>
Dry over H <sub>2</sub> SO <sub>4</sub> .....	48 hrs.	Drying unnecessary	
Manipulation.....	5 min.		
Set up and extract.....	2 hrs.	Set up, and extract	
Manipulation.....	5 min.	50 min.....	10 times..... 1 hr.
Remove samples, grind, replace, re-extract.....	2 hrs.	Grinding with solvent is included in procedure	
Manipulation.....	30 min.		
Evaporate solvent, heat to constant weight.....	2 hrs.	Evaporate solvent, heat 3 hrs... to constant weight... 3 hrs.	
Total time.....	54 hrs. 40 min.	3 hrs. 50 min.	4 hrs.
Manipulation ... ..	40 min.	50 min.	1 hr.

## REPORT ON NAVAL STORES.

By F. P. VEITCH (Bureau of Chemistry, Washington, D. C.), *Referee*.

This is merely a report of progress.

Before undertaking any cooperative work, it was found necessary to study the literature concerning methods for the determination of melting point that might be applicable to rosin, and subsequently, to do some preliminary work with what appeared to be the most suitable of these methods before deciding which should be studied collaboratively.

It was not until about a month ago that two methods for this purpose were finally decided upon. These may be designated "The Capillary Tube Method" and the "Drip Method"; they are described as follows:

### INSTRUCTIONS FOR THE DETERMINATION OF SOFTENING AND MELTING POINTS OF ROSIN.

#### *Capillary Tube Method.*

#### DEFINITIONS.

*Softening Point.*—The temperature at which powdered rosin in a melting point tube begins to darken and coalesce.

*Melting Point.*—The temperature at which powdered rosin in a melting point tube loses its powdered or crystalline shape and becomes wholly transparent.

#### APPARATUS.

(a) *Thermometers.*—Must be accurate, graduated from 45°–100°C. and have a length of about 5½ inches (137 mm.) and a diameter of about ¼ inch (6 mm.).

(b) *Test tubes.*—Should be ¾ by 6 inches (20 by 150 mm.), fitted with corks grooved at sides to permit air circulation and bored at center to receive thermometer.

(c) *Melting point tubes.*—Should be of thin glass, inside diameter 1/16 inch (1.5 mm.), length about 2 inches (50 mm.), and closed at one end.

(d) *Flasks.*—Ordinary Erlenmeyer type of 300 cc. capacity, the mouths of which must be at least 1 inch (25 mm.) in diameter, fitted with a cork grooved at sides to permit air circulation, and bored at center to receive test tube.

#### DETERMINATION.

Place 250 cc. of glycerin in the flask, insert the grooved cork carrying the test tube so that its lowest point is  $\frac{1}{4}$  inch above the bottom of the flask, and heat the flask so that the thermometer rises at the rate of 1°C. per minute above 50°C.

From the freshly broken surface of a lump of the rosin quickly powder a sample (1–2 grams) and *at once* fill four melting point tubes to a depth of about 10 mm., lightly packing with a fine glass rod or by tapping. Pass the stem of the thermometer through a cork, grooved to permit air circulation. Attach two of the filled melting point tubes to the thermometer so that the rosin is opposite the bulb. When the temperature of the glycerin in the flask reaches 45°C., place the thermometer and tubes in the test tube suspended in the flask so that its lowest point is  $\frac{1}{4}$  inch above the bottom of the test tube. Continue to heat the flask at the rate of 1°C. per minute, and note and record the temperature at which the rosin begins to darken and coalesce (softening point) and also the temperature at which the rosin loses its powdered or crystalline appearance and becomes wholly transparent (melting point). It is necessary during a test to have a light behind the flask, daylight or electric light. Repeat the test, using the other two filled melting point tubes and making sure that the glycerin bath is below 45°C. at the start.

The four tests of melting point must not vary more than 1°C.; otherwise two additional tests must be made and the average reported. The softening point tests will probably vary more than the melting point tests. Report the average of each set of results as the “softening point” and “melting point”, respectively

#### INSTRUCTIONS FOR THE DETERMINATION OF MELTING POINT OF ROSIN.

##### *Drip Method.*

##### DEFINITION.

*Melting Point.*—The temperature at which the elongated drop of rosin from the thermometer bulb touches the bottom of the test tube.

##### APPARATUS.

(a) *Thermometers.*—Use an A. S. T. M. Flash Test Thermometer; range  $-5^{\circ}$ – $+110^{\circ}$ C.; total length, 277–282 mm.; bulb length, 9–13 mm. and diameter not greater than 6–7 mm.

(b) *Test tubes.*—Should be 22 by 180–200 mm., fitted with corks grooved at sides to permit air circulation and bored at center to receive thermometer.

(c) *Beakers.*—Low or ordinary 600 cc.

#### DETERMINATION.

From the freshly broken surface of the rosin, remove a sufficient quantity to fill a 15 mm. diameter test tube to a depth of about 40 mm. Place the test tube in a boiling water bath until the rosin becomes fluid enough to put on the thermometer. Heat the thermometer to 100°C. and dip into the rosin to a depth of 15 mm. from the bottom of



the bulb, securing a coating that weighs when cold 0.50–0.55 gram and is uniformly distributed. (It will probably be necessary to shape the rosin with the fingers.) Cool the film rapidly, taking care that it remains evenly distributed, and allow to rest 10 minutes after cooling to room temperature. Fill the beaker to a depth of 90 mm. with water at not over 45°C. Pass the thermometer through the cork and insert in the test tube so that the lowest point is 25 mm. above the bottom of the test tube. Fix the test tube in the beaker of water so that the lowest point of the test tube is 25 mm. above the bottom of the beaker. Heat the water so that the thermometer rises not more than 2°C. per minute up to 65°C. and at a rate of 1°C. per minute above 65°C. The average temperature in four tests during which the rosin dropping from the bulb of the thermometer touches the bottom of the test tube is recorded as the melting point (drip method). If the variation of the four determinations is greater than 1°C., make an additional determination and report the average of the five determinations as the melting point (drip method).

The preceding methods, together with two samples of rosin, were sent out to twelve chemists who, even at so late a date, kindly consented to do some work with them.

Up to the time of the meeting five chemists had reported their results, and it is anticipated that several more will be heard from within the next ten days.

The results so far obtained are given in the table.

*Results of determining melting point of rosin, using two methods.*

COLLABORATORS	CAPILLARY TUBE METHOD				DRIP METHOD	
	Sample M		Sample E		Sample M	Sample E
	Softening point	Melting point	Softening point	Melting point	Melting point	Melting point
R. H. Smith A. & M. College Miss.	*	*	*	*	78.0	79.6
R. C. Palmer Newport Co. Pensacola, Fla.	57.1	59.1	60.6	63.6	79.3	81.9
Bureau of Standards Washington, D. C.	60.2	65.0	59.5	66.5	77.75	79.75
W. C. Smith Bureau of Chemistry Washington, D. C.	59.3	69.0	60.7	70.6	79.0	80.0
W. K. Ward, Jr. Bureau of Chemistry Washington, D. C.	59.6	68.6	58.4	71.8	79.4	80.6
Highest	60.2	69.0	60.7	71.8	79.4	81.9
Lowest	57.1	59.1	58.4	63.6	77.75	79.6

\* Could not be accurately determined.

## DISCUSSION.

It will be seen from these results that by the capillary tube method the figures for the softening point on both samples are very satisfactory. The maximum and minimum figures for true melting point, however, are decidedly wide and can not be regarded as satisfactory. Since, however, this is probably the first time the analysts have used these methods, it is believed that more concordant results will be obtained upon greater familiarity with them.

The results by the so-called drip method on both samples are most excellent and leave nothing to be desired. The difference between the highest and lowest results is less than 2 degrees on both rosins. However, it must be pointed out that the melting point temperature by the drip method is approximately 10 degrees higher than by the capillary tube method. The drip method can not be called a true melting point method. It is, in fact, more a yielding or softening point method than a melting point method.

It remains to be determined by future work and discussion which of these procedures is to be preferred.

## RECOMMENDATION.

It is recommended that further study on a larger number of samples be conducted with the capillary tube method and the drip method, and also by any other method that may promise to yield concordant and informing results.

## CONTRIBUTED PAPERS.

## SOME ANALYSES OF COMMERCIAL CORN SIRUPS.

By C. P. LATHROP<sup>1</sup> (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.).

The presence of varying amounts of commercial corn sirup in manufactured jellies, jams, and preserves has been a disturbing factor in the correct interpretation of the analytical results on this class of commercial products. This is largely due to the fact that commercial corn sirup, unlike the sucrose that it replaces, is a liquid mixture of variable composition containing as its principal constituents varying amounts of dextrose, dextrin, and maltose, each of which has a widely different specific rotatory value. In addition, corn sirup contains other ingredients incident to the process of manufacture.

A previous study of the composition of American commercial corn sirups was published by Bryan<sup>2</sup> in 1911. The work here reported was undertaken for the purpose of determining the analytical differences, if any, between the present day corn sirups and those reported by Bryan. The samples were secured in 1923 direct from different corn sirup manufacturers, and they are representative of the product manufactured in the United States for use in jellies and jams. The methods of analysis used and the analytical results obtained are given in the table.

The results shown in the table indicate that the samples are of a general uniform composition. The ash content is materially lower than that reported by Bryan, while the moisture, reducing sugars, and polarization constants are very similar. The invert polarization of the normal solution at 87°C. divided by 163 (the average found by Bryan and the factor previously recommended by A. E. Leach<sup>3</sup>) results in the approximate percentage of commercial corn sirup. To find the corresponding percentage of corn sirup solids, this factor, 163, must be replaced by 195.8, as indicated by Bryan's work, or by 196.2, as indicated by the work here reported. The corn sirup ash consists chiefly of chlorides. Compared with the ash of fruits, the  $P_2O_5$  content and the alkalinity number are quite similar, while the  $K_2O$  content is much less. A comparison of the moisture and corresponding total solids by the Abbé refractometer and vacuo drying at 70°C. shows a fairly close agreement. The refractometer method gave uniformly slightly higher results.

The convention adjourned at 5 o'clock, Wednesday, October 22nd. The proceedings for Wednesday morning and afternoon were published in Volume VIII, No. 3.

<sup>1</sup> Present address: National Preservers' Association, Washington, D. C.

<sup>2</sup> *J. Franklin Inst.*, 1911, 172: 337.

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 81, 73.

Composition of commercial glucose.

SAMPLE NO.	BAUMÉ <sup>1</sup>	BY REFRACTOMETER		BY VACUO-DRYING 70°C.		SUGARS BEFORE INVERSION AS GLUCOSE <sup>4</sup> (BY COPPER) <sup>5</sup>	SUGARS AFTER INVERSION AS GLUCOSE <sup>4</sup> (BY COPPER) <sup>5</sup>	SUGAR AFTER 2 <sup>1</sup> HRS. BOILING IN DILUTE HCl AS GLUCOSE <sup>5</sup> (BY COPPER) <sup>5</sup>	TOTAL ASH <sup>6</sup>	ALKALINITY NO. OF ASH <sup>7</sup>	SULFUR IN ASH <sup>7</sup> per cent	P <sub>2</sub> O <sub>5</sub> IN ASH <sup>7</sup> mg. per 100 grams	K <sub>2</sub> O IN ASH <sup>8</sup> per cent	Cl IN ASH <sup>8</sup> per cent	ACIDITY <sup>10</sup> % N/10 acid	POLARIZATIONS N/1 SOLUTION			COMMERCIAL GLUCOSE FACTOR 163	COMMERCIAL GLUCOSE 196.17 <sup>12</sup> per cent
		Moisture <sup>3</sup> per cent	Total solids <sup>3</sup> per cent	Moisture <sup>3</sup> per cent	Total solids <sup>3</sup> per cent											Before inversion 20°11	After inversion 20°11	After inversion 87°11		
Inv. 46818	44.17	15.62	84.38	17.08	82.92	34.98	35.81	83.52	0.287	10.5	3.99	29.7	0.61	24.40	4.0	+178.2	+176.8	+168.0	101.8	85.64
Inv. 46918	43.42	17.83	82.17	18.57	81.43	35.25	36.34	79.76	0.269	10.6	4.24	29.6	0.79	25.90	4.0	+172.8	+169.6	+160.8	98.6	81.97
Inv. 48318	42.98	18.50	81.50	19.70	80.30	35.49	36.40	81.16	0.314	10.8	2.39	28.9	1.79	21.08	8.0	+169.6	+167.6	+157.0	96.3	80.03
Inv. 45256	43.02	18.58	81.42	19.51	80.49	37.37	37.59	81.28	0.290	11.7	2.37	26.9	2.14	21.18	8.0	+166.2	+163.6	+153.0	93.9	78.00
Inv. 48317	43.03	18.45	81.55	19.58	80.42	36.65	37.36	81.20	0.301	13.5	2.68	16.7	2.64	25.31	5.3	+167.2	+165.6	+155.4	95.3	79.22
Inv. 45818	43.63	16.73	83.27	18.34	81.66	35.33	36.01	81.56	0.318	11.4	5.03	17.7	1.80	18.49	6.7	+175.0	+173.4	+163.2	100.1	83.19
Inv. 46916	43.00	18.45	81.55	19.58	80.42	32.95	33.11	81.04	0.409	9.8	4.25	23.0	1.49	21.03	6.7	+175.0	+173.4	+160.4	98.4	81.76
Inv. 46917	42.89	18.58	81.42	19.55	80.45	35.64	35.85	80.96	0.323	10.6	3.34	21.7	3.48	22.79	6.7	+168.6	+167.6	+156.8	96.2	79.93
Inv. 48319	43.58	17.03	82.97	18.63	81.37	37.20	38.15	82.00	0.381	14.7	1.54	16.9	3.65	18.30	5.3	+170.0	+167.4	+158.4	97.2	80.75
Average 9 samples	43.30	17.75	82.25	18.94	81.05	35.65	36.28	81.40	0.321	11.5	3.31	23.4	2.04	22.05	6.1	+171.4	+169.4	+159.0	97.50	81.05
Moisture-free basis vacuo drying 70°C.	.	...	....	.	100.00	43.98	44.76	100.43	0.396	11.5	3.31	28.9	2.04	22.05	7.5	+211.5	+209.1	+196.17	118.5	100.00

<sup>1</sup> Assoc. Official Agr. Chemists, Methods, 1925, 179, (6a).<sup>2</sup> Ibid., (7).<sup>3</sup> Drying on asbestos, 8-10 hrs. in vacuo at 70°C.<sup>4</sup> Assoc. Official Agr. Chemists, Methods, 1925, 190, (34, 35).<sup>5</sup> Ibid., 116, (21).<sup>6</sup> Ibid., 116, (7).<sup>7</sup> Ibid., 211, (9, 10); 3, (8).<sup>8</sup> Ibid., 14, (45a).<sup>9</sup> Ibid., 44, (13).<sup>10</sup> Ibid., 213, (16).<sup>11</sup> Ibid., 186, (23).<sup>12</sup> Factor =  $\frac{1}{100} \times \frac{\text{Total Solids}}{\text{Total Solids}} = \frac{159.00}{81.05}$

## CATAWBA GRAPE JUICE.

By B. G. HARTMANN (Food Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

U. S. Department of Agriculture Bulletin No. 656 discusses the manufacture and chemical composition of commercial Concord grape juice. The following pages are devoted to a similar study of its counterpart, Catawba grape juice. In addition to the information on manufacture and composition the paper includes a discussion of the significance of the analytical data as a means of recognizing adulteration and also a discussion of methods for the determination of added water and sugar in Catawba grape juice.

The Bureau of Chemistry has published a number of bulletins on the chemical composition of native grapes, one of which<sup>1</sup> includes results on a large number of samples of Catawba grapes harvested in 1908-1911 and 1913. The data obtained on these samples are confined to juices obtained by hand pressure and are not, therefore, comparable with data obtained on juices extracted commercially at high pressure. Furthermore, the chemical work on these samples is limited in the main to the determination of acid and sugar. Although the acid-sugar ratio of a grape is of first importance in making a choice of material suitable for grape juice manufacture it is of little value as a means for judging purity. Because of the paucity of analytical data necessary for the detection of adulterants in commercial grape juices, the investigation hereinafter recorded was undertaken, in the hope that it would lead to information that would be of assistance in detecting improper manufacturing practices.

## THE CATAWBA GRAPE.

The Catawba grape is native to the Eastern and Central States, growing chiefly along the shores of Lake Erie in northern Ohio and the central lakes of New York. Its origin is obscure. In seniority, however, it has been conceded first place over all the other varieties of our native grapes. The species to which the Catawba belongs is uncertain. Most probably it is *labrusca*, mixed with *vinifera*. The berry is medium in size, rather thick skinned, and dull purplish red in color. The flavor of the flesh is pleasantly vinous, indicative of *vinifera* blood, with just an indication of foxiness.

That the Catawba has been given preference over other native varieties of grapes for wine making, especially for the production of champagne, speaks highly of its quality. However splendid its quality and flavor, it

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<sup>1</sup> U. S. Dept. Agr. Bull. No. 452, 17.

has not prospered as a competitor of the Concord grape. This is evidenced by the fact that the acreage devoted to the production of the two varieties is overwhelmingly in favor of the Concord. The Concord is a mid-season variety, while the Catawba matures very much later in the fall. The Concord possesses a strong flavor, and when heated it produces a richly colored juice, qualities desirable for grape juice production that are lacking in the Catawba.

The Catawba is preeminently a wine grape, while the Concord is more suited for grape juice and beverage purposes. Notwithstanding these apparent deficiencies, the Catawba, when properly matured, produces a juice that is highly esteemed for its delicate flavor.

#### MANUFACTURE OF CATAWBA GRAPE JUICE.

The basic principles underlying the manufacture of red and white grape juice are essentially the same; the only difference is that in the manufacture of red grape juice the fruit is heated to draw color, whereas in the production of white juice this operation is omitted.

For the manufacture of Catawba juice the grapes are pressed unstemmed. The juice is strained through burlap as it runs from the presses, collected in steam jacketed kettles, and heated to about 190°F. From the heating kettles it is immediately drawn off for clarification into the storage vessels. After several months the juice is filtered, filled into trade packages, and pasteurized at about 170°F. The heating and subsequent pasteurization give the juice a brownish color and a slight cooked taste that is caused by caramelization of sugar. To avoid caramelization and to preserve the delicate flavor of the fruit, another method of preservation, commonly known as the "sulfuring" process, is practiced. In this process the juice running from the presses is treated in the cold with anhydrous potassium or sodium bisulfite (360 grams to 100 gallons of juice) before it is stored for clarification. After several months' storage the juice is drawn off into the trade packages and is distributed without being pasteurized. The sulfur dioxide, besides preserving the juice, exercises a bleaching effect, thereby producing a juice that is almost water white.

#### EXPERIMENTAL WORK.

The principal object of the investigation was to secure data on the chemical composition of pure commercial Catawba grape juice. Thirty samples of typical commercial juices were collected. They were prepared under the direct supervision of the writer and analyzed by him in the Food and Drug Inspection Laboratory of the U. S. Department of Agriculture at Chicago. In their production no additions or abstractions were made other than those occurring in the normal process of manufacture. The fruit was grown on the Bass Islands, situated in Lake Erie north of Sandusky, Ohio, during the seasons of 1914-1917, inclusive.

TABLE 1.  
*Chemical composition of pasteurized juices.*

Season	1914												1916			Max.	Min.	Ave.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
Sample Number																		
Alcohol by volume	0.09	0.20	0.28	0.12	0.18	0.08	0.20	0.15	0.18	0.10	0.15	0.15	0.10	0.10	0.15	0.28	0.08	0.15
Total solids	19.94	19.52	19.52	20.44	19.68	20.10	19.81	19.89	20.00	18.90	18.53	18.11	18.90	23.57	22.38	23.57	18.11	19.95
Non-sugar solids	2.17	2.12	2.27	2.17	2.09	1.90	2.39	2.22	2.00	3.27	3.28	3.15	3.27	2.97	2.96	3.27	1.90	2.55
Sugar as invert, direct	17.77	17.40	17.25	18.27	17.59	18.20	17.42	17.67	18.00	15.63	15.27	14.96	15.63	20.60	19.42	20.60	14.96	17.40
Sugar as invert, after inversion	17.72	17.51	17.37	18.30	17.51	18.10	17.37	17.60	17.97	15.52	15.12	14.83	15.54	20.60	19.47	20.60	14.83	17.37
Polarization, undiluted*: Direct at 20°C. Ventzke	26.0	26.0	25.8	27.2	25.5	28.0	27.6	28.1	28.7	21.2	20.4	22.6	22.0	29.5	28.8	29.5	20.4	25.8
Invert at 20°C. Ventzke	26.5	26.0	26.0	27.5	26.5	29.0	28.0	29.0	29.0	20.8	21.6	22.4	22.4	30.0	28.0	30.0	20.8	26.2
Invert at 87°C. Ventzke	7.5	7.0	6.0	7.0	6.5	6.0	7.5	7.5	7.5	4.9	5.0	5.2	5.0	6.0	5.5	7.5	4.9	6.3
Total acidity as tartaric	0.96	1.03	1.01	0.99	1.02	0.93	0.99	0.93	0.91	1.49	1.53	1.49	1.45	1.30	1.25	1.53	0.91	1.15
Volatile acid as acetic	0.02	0.04	0.02	0.02	0.02	0.02	0.04	0.02	0.01	0.01	0.03	0.03	0.01	0.01	0.01	0.04	0.01	0.02
Fixed acid as tartaric	0.93	0.98	0.98	0.96	0.99	0.90	0.94	0.90	0.90	1.48	1.49	1.45	1.44	1.29	1.24	1.49	0.90	1.12
Total tartaric acid	0.78	0.79	0.79	0.78	0.79	0.74	0.77	0.76	0.77	0.87	0.89	0.81	0.84	0.86	0.86	0.89	0.74	0.81
Free tartaric acid	0.28	0.29	0.30	0.27	0.28	0.28	0.30	0.28	0.25	0.34	0.38	0.38	0.32	0.30	0.33	0.38	0.25	0.31
Cream of tartar	0.50	0.50	0.50	0.51	0.51	0.48	0.49	0.52	0.55	0.53	0.53	0.44	0.53	0.56	0.52	0.56	0.44	0.51
Ash	0.30	0.29	0.29	0.28	0.29	0.26	0.30	0.30	0.30	0.33	0.35	0.32	0.33	0.33	0.33	0.35	0.26	0.31
Alkalinity, water-soluble ash, cc. 0.1 N acid per 100 cc.	26.8	26.8	26.8	27.0	27.2	25.6	25.8	27.4	29.0	28.4	28.4	23.2	28.0	29.6	27.6	29.6	23.2	27.2
Alkalinity, water-insoluble ash, cc. 0.1 N acid per 100 cc.	6.4	6.4	6.0	7.0	6.6	5.2	5.4	5.0	6.0	7.2	5.6	5.6	6.4	7.6	7.6	7.6	5.0	6.3
Total P <sub>2</sub> O <sub>5</sub> , Mg. per 100 cc.	19.8	25.1	24.8	24.2	23.3	21.2	24.2	23.9	23.4	27.3	32.7	31.1	29.3	29.4	27.7	32.7	19.8	25.8
Acid-sugar ratio (1 : X)	18.5	16.9	17.1	18.5	17.2	19.6	17.6	19.0	19.8	10.5	10.0	10.0	10.8	15.8	15.5	19.8	10.0	15.8

\* All polarizations are levorotatory.

TABLE 2.  
Chemical composition of sulfured juices\*.

Season	1914				1915				1916				1917						Max.	Min.	Ave.
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30						
Sample Number																					
Alcohol by volume	0.20	0.32	0.45	0.20	0.25	0.25	0.15	0.15	0.15	0.20	0.14	0.20	0.20	0.20	0.20	0.45	0.14	0.22			
Total solids	20.05	19.10	17.67	18.03	17.69	22.43	22.86	22.86	23.09	20.02	20.05	19.68	19.89	19.26	19.26	23.09	17.67	20.13			
Non-sugar solids	2.05	1.93	3.12	2.91	2.73	2.80	2.89	2.72	2.82	3.09	3.10	3.11	3.14	2.88	3.06	3.14	1.93	2.82			
Sugar as invert, direct	18.00	17.17	14.55	15.12	14.96	19.63	19.97	20.14	20.27	16.93	16.95	16.57	16.75	16.38	16.20	20.27	14.55	17.31			
Sugar as invert, after inversion	17.98	17.14	14.52	15.15	14.85	19.68	19.94	20.18	20.28	16.89	16.95	16.52	16.71	16.39	16.17	20.28	14.52	17.29			
Polarization, undiluted†:																					
Direct at 20°C. Ventzke	26.6	26.2	19.8	22.2	22.0	29.0	29.8	30.0	29.5	24.8	25.6	25.2	25.6	25.6	24.8	30.0	19.8	25.8			
Invert at 20°C. Ventzke	27.0	26.5	20.0	22.4	22.4	29.0	31.0	31.0	30.0	25.4	26.4	25.6	25.6	24.6	25.6	31.0	20.0	26.2			
Invert at 87°C. Ventzke	6.0	6.5	5.3	5.3	5.0	6.2	6.0	6.0	6.5	4.8	4.0	5.6	4.0	6.4	6.4	6.5	4.0	5.6			
Total acidity as tartaric	1.03	1.02	1.39	1.39	1.37	1.31	1.30	1.27	1.28	1.65	1.63	1.61	1.58	1.59	1.61	1.65	1.02	1.40			
Volatile acid as acetic	0.06	0.07	0.12	0.04	0.07	0.05	0.04	0.05	0.03	0.03	0.04	0.04	0.03	0.05	0.02	0.12	0.02	0.05			
Fixed acid as tartaric	0.95	0.93	1.24	1.34	1.28	1.25	1.25	1.21	1.24	1.61	1.58	1.56	1.54	1.53	1.59	1.61	0.93	1.34			
Total tartaric acid	0.65	0.65	0.57	0.62	0.61	0.77	0.79	0.80	0.78	0.86	0.82	0.83	0.83	0.83	0.80	0.86	0.57	0.75			
Free tartaric acid	0.24	0.24	0.07	0.21	0.21	0.23	0.29	0.27	0.26	0.36	0.31	0.33	0.34	0.36	0.31	0.36	0.07	0.27			
Cream of tartar	0.41	0.40	0.49	0.39	0.38	0.50	0.47	0.50	0.49	0.47	0.47	0.47	0.48	0.45	0.47	0.50	0.38	0.46			
Ash	0.28	0.28	0.35	0.28	0.27	0.33	0.33	0.30	0.30	0.29	0.28	0.28	0.28	0.27	0.29	0.35	0.27	0.29			
Alkalinity, water-soluble ash, cc. 0.1 N acid per 100 cc.	21.6	21.2	26.0	20.8	20.4	26.8	25.2	26.8	26.0	24.8	25.2	24.8	25.6	23.6	24.8	26.8	20.4	24.2			
Alkalinity, water-insoluble ash, cc. 0.1 N acid per 100 cc.	5.8	6.0	7.2	6.4	6.4	9.2	8.0	8.4	8.4	8.4	9.2	8.4	7.2	8.0	7.6	9.2	5.8	7.6			
Total P <sub>2</sub> O <sub>5</sub> , Mg. per 100 cc.	22.7	23.8	30.4	30.4	30.6	29.5	30.1	27.9	28.2	30.5	29.3	29.1	26.7	28.5	31.6	31.6	22.7	28.6			
Acid-sugar ratio (1 : X)	17.5	16.8	10.5	10.9	10.9	15.0	15.4	15.9	15.9	10.3	10.4	10.3	10.6	10.3	10.1	17.5	10.1	12.7			

\* 1914 and 1915 juices preserved with potassium bisulfite; 1916 and 1917, with sodium bisulfite.

† All polarizations are levorotatory.



In the course of the work the composition as to acid and solid content of juices running from the presses at different stages of the pressing operation was studied. The following data were obtained, expressed in grams per 100 cc.:

	NO. 1	NO. 2	SERIES NO. 3
Free run—Acid, as tartaric.....	0.92	0.80	0.87
Solids.....	18.0	18.9	18.6
Press $\frac{1}{2}$ down—Acid, as tartaric.....	1.22	1.08	1.14
Solids.....	17.9	18.9	18.5
Press down—Acid, as tartaric.....	1.31	1.20	1.23
Solids.....	17.7	18.8	18.5

The results show only slight differences in the solids content of the juices obtained at the various pressures. There is, however, a great difference in the acid content of the free run and final juice.

Tables 1 and 2 record the data obtained on the thirty juices. The results are expressed in grams per 100 cc. unless otherwise stated.

In the course of this investigation a number of determinations were made, the results of which are not incorporated in Tables 1 and 2. These results are shown in Table 3.

TABLE 3.  
*Additional determinations.*  
(Results expressed in grams per 100 cc. of juice.)

Season	PASTEURIZED JUICES				SULFURED JUICES							
	1915		1916		1915		1916		1917			
Sample Number	10	11	14	15	18	19	21	22	25	27	29	30
Nitrogen	0.056	0.063	0.030	0.031	0.060	0.059	0.030	0.029	0.049	0.048	0.045	0.053
Tannin and Color- ing Matter	0.058	0.058	0.074	0.072	0.055	0.065	0.074	0.068	0.056	0.058	0.056	0.058
Calcium-oxide (CaO)	0.014	0.010	0.011	0.012	0.011	0.012	0.010	0.010	0.012	0.010	0.012	0.014
Magnesium-oxide (MgO)	0.014	0.013	0.014	0.016	0.013	0.019	0.015	0.015	0.013	0.012	0.013	0.017
Potassium-oxide (K <sub>2</sub> O)	0.184	0.187	0.151	0.137	0.176	0.139	0.120	0.121	0.119	0.124	0.117	0.120
Sodium-oxide (Na <sub>2</sub> O)	0.005	0.008	0.007	0.008	0.005	0.004	0.024	0.025	0.030	0.031	0.027	0.027
Sulfur-trioxide (SO <sub>3</sub> )	0.028	0.041	0.022	0.028	0.048	0.047	0.031	0.037	0.026	0.026	0.030	0.036
Sulfur-dioxide (SO <sub>2</sub> )	0.003	0.003	0.004	0.003	0.095	0.030	0.026	0.025	0.025	0.030	0.028	0.026
Chlorine (Cl)	0.003	0.002	0.003	0.003	0.003	0.003	0.063	0.003	0.002	0.003	0.003	0.004

The methods used were those for wines described in *Official and Tentative Methods of Analysis*<sup>1</sup>, so far as they applied and with only such modifications as made them suitable for the unfermented products.

#### DISCUSSION OF ANALYTICAL DATA.

The variation in chemical composition of the juices of the different seasons is striking. Especially is this true of the acid and sugar content as indicated by the ratios of these ingredients. It is noted, however, that for the same seasons the ratios are very similar.

Catawba grape juice contains very considerable amounts of free tartaric acid, differing in this respect from Concord grape juice. The analytical data also indicate the presence of a considerable amount of free acid, other than tartaric, which is not accounted for. As has been demonstrated by E. K. Nelson<sup>2</sup> of this Bureau, the Concord grape contains large quantities of malic acid. In fact, his investigation has shown that malic acid predominates over the other acids of the grape. While no work has been done to show that this condition also obtains in the Catawba grape, it may be assumed from general knowledge regarding the acid content of grapes that such is the case.

The polarization of the inverted solution at 87°C. is levorotatory, signifying an excess of levulose over dextrose, a normal condition for the maturing grape. As the grape ripens the total acidity decreases and the levulose increases so that the relation between the total acidity and the polarization of the inverted solution at 87°C. is in a measure indicative of the degree of maturity. Because of this inverse relation of polarization to acidity, a juice showing a low acidity and low polarization at 87°C. is immediately under suspicion of having been watered.

Because pure Catawba juices are practically free from sucrose, the addition of sucrose to a juice should be readily recognized, but unfortunately the inversion that occurs owing to the relatively high acid content of the juice tends to destroy the evidence of such an addition.

A properly prepared Catawba grape juice contains only small amounts of alcohol. Only one sample of the thirty examined contained more than one-third per cent of alcohol by volume, or 0.25 per cent by weight.

Of all the determinations reported in Tables 1 and 2, the cream of tartar, ash, and the alkalinity of the water-soluble ash show the least tendency to variation. Theoretically, this condition is to be expected since Catawba grape juice is saturated with cream of tartar, and the ash and alkalinity of the water-soluble ash are directly related to the cream of tartar content.

The chemical data in Table 3 require no discussion. For the purpose of the detection of adulteration they are of little value. Attention is

<sup>1</sup> *Assoc. Official Agr. Chemists, Methods*, 1925, 361.

<sup>2</sup> *J. Am. Chem. Soc.*, 1925, 47: 1177.

directed, however, to the results for sulfur dioxide and sulfur trioxide. It is interesting to note that for the preservation of a grape juice 300 mg. of sulfur dioxide per liter is a safe limit, and also that practically no oxidation of sulfur dioxide to sulfur trioxide takes place. A calculation based upon the available sulfur dioxide of the bisulfite added and the sulfur dioxide retained in the finished juice indicates a loss of sulfur dioxide of approximately 50 per cent. It is also noted that the ash of the pasteurized juices consists of about 75 per cent of potassium carbonate.

The preceding discussion of the results obtained during this investigation indicates that for the purpose of judging the purity of a Catawba grape juice in cases of minor adulterations they are of little value. However, as adulterations become more pronounced, the significance of some of the data gains in weight. Fortunately, the adulterations to which a Catawba grape juice is subject are of a restricted nature, being confined chiefly to additions of water and sugar, and in rare instances to the addition of methylantranilate.

According to Power and Chesnut<sup>1</sup>, methylantranilate is not a normal constituent of the Catawba grape, so that in the event its presence in the juice is suspected a qualitative test will suffice to determine its addition.

#### DETERMINATION OF ADDED SUGAR SOLUTION (WATER) IN CATAWBA GRAPE JUICE.

In considering methods for the determination of water in grape juice, the writer's attention was called to a procedure employed by Tissier and Francois<sup>2</sup> for determining added water in wines. The method used by these investigators is based on the premise that a normal wine is saturated with cream of tartar and that the dissolving power of a watered wine for cream of tartar is increased in proportion to the amount of added water that the wine contains. As it was considered that the principle laid down by Tissier and Francois should be applicable to Catawba grape juice—since with respect to saturation with cream of tartar a Catawba grape juice and a wine may be considered analogous—it was decided to use it as the basis for a method. After considerable experimentation a procedure was formulated. A brief description of the development of the method and the theoretical considerations involved follows: In the manufacture of a Catawba grape juice the grapes are pressed cold, and it may be assumed that the juice running from the presses is saturated with cream of tartar<sup>3</sup>. The temperature at which a grape juice is stored

<sup>1</sup> *J. Agr. Research*, 1923, 23: 50.

<sup>2</sup> *Ann. fals.*, 1914, 7: 251-4.

<sup>3</sup> This assumption is apparently contradicted by the varying cream of tartar content of the juices as shown in Tables 1 and 2. However, it must be borne in mind that the quantity of cream of tartar necessary for the saturation of a grape juice depends upon a number of factors, among which may be mentioned the varying quantities of sugar, malic acid, etc., in the juices.

in the cellar of the factory for the purpose of clarification is about 15°C., which temperature is about 10° under that ordinarily prevailing in the laboratory. Obviously then, when a factory juice reaches laboratory temperature it will not be completely saturated with cream of tartar. It was found that a factory made juice (held at 15°C. for several months) requires from 90–100 mg. of cream of tartar per 100 cc. of juice to re-establish saturation at 25°C., the average of four determinations being 95 mg.

It was also found that the quantity of cream of tartar that a watered juice will dissolve is practically proportional to the quantity of added water present in the juice. Experiments in this direction showed that the addition of 10 per cent by volume of a 20 per cent sugar solution to grape juice increases the dissolving power for cream of tartar approximately 60 mg. per 100 cc. at a temperature of 25°C. From these two values, together with “*a*”, the acidity of the original juice, and “*b*”, the acidity of the juice after saturation with cream of tartar at 25°C. (expressed in terms of 0.1 *N* sodium hydroxide per 100 cc.) it is possible to calculate (*W*), the percentage by volume of added sugar solution in a given juice.

$$W = \frac{0.0188 (b - a) - 0.095}{0.006}.$$

Because of the difficulty of maintaining a temperature of 25°C. for the prolonged period of time required to effect complete saturation, a corrective factor was determined; its introduction in the formula would take care of the small variations from the initial temperature. It was found that the dissolving power of 100 cc. of pure juice for cream of tartar increased approximately 25 mg. for each degree over 25°C. Introducing this factor into the formula, it is shown that—

$$W = \frac{0.0188 (b - a) - 0.095 - 0.025 \left( \frac{t^{\circ} - 25}{2} \right)}{0.006}.$$

*W* = Percentage by volume of added water (20 per cent sugar solution);

*b* = Acidity of treated juice, cc. 0.1 *N* sodium hydroxide per 100 cc.;

*a* = Acidity of original juice cc. 0.1 *N* sodium hydroxide per 100 cc.;  
and

*t*° = Temperature after shaking.

In factory practice the addition of water to a juice is generally made in the form of a 20 per cent sugar solution so that it seemed preferable to express the percentage of dilution in terms of sugar solution rather

than as water. Hence "W" in the formula denotes 20 per cent sugar solution and not percentage of water. To obtain the actual amount of water added to a juice it would be necessary to make allowance for the sugar content of the sugar solution used for the dilution. It is not believed, however, that such correction is necessary, since it would only be a small one, and also because the determination, as outlined, is merely an approximation.

Concerning the method of saturating the sample with cream of tartar, it may be stated that experiments showed that one hour in a rotating shaker (16 rotations per minute) is sufficient to effect complete saturation of the juice. In order to maintain the temperature as nearly as possible at 25°C. the precaution of wrapping the container (Mason jar) in several thicknesses of heavy paper before placing it in the shaking machine was taken. It was found that by so doing the temperature could be controlled within one degree either side of 25°. The following method was finally worked out.

#### METHOD.

Transfer about 50 cc. of the filtered juice to a 2 ounce tincture bottle containing ten pieces of glass rod about 15 mm. long and 5 mm. in diameter, and approximately one gram of finely powdered cream of tartar. Cork the bottle tightly and place it neck downward in a pint Mason jar. Fill the Mason jar with water of 25°C. and hold at this temperature for one-half hour, shaking occasionally. (It was found that by placing the Mason jar containing the sample in a bucket of water the temperature of 25°C. could be more readily controlled.) After this time, seal the Mason jar; wrap it in three sheets of heavy wrapping paper, making three separate wrappings; place in a mechanical shaker; and shake for one hour. After the shaking has been accomplished, ascertain the temperature, "*t*", of the water in the Mason jar. Filter the juice immediately and titrate 10 cc. with 0.1*N* sodium hydroxide, using phenolphthalein as indicator. Repeat the titration with 10 cc. of the original juice. Make the titrations side by side in order to obtain the same shade of pink with the greatest possible accuracy. For calculating the percentage of added sugar solution (water) use the formula given previously.

Pure factory juices examined by this method indicate a quantity of added water varying from 1-3 per cent. Such a quantity may well be introduced in normal factory practice, through the use of water in the washing of apparatus.

The method was tried on juices prepared by diluting pure factory juice with known quantities of sugar solution and also on commercial juices obtained in the market. The results obtained were very satisfactory.

#### ESTIMATING THE ADDED SUGAR IN CATAWBA GRAPE JUICES.

As has been said, the addition of water to a grape juice is made in the form of a sugar solution. In factory practice the sugar solution used for

the purpose of reducing acidity is prepared by dissolving two pounds of cane sugar in one gallon of water. A sugar solution prepared in this manner has a sugar content of slightly less than 20 per cent. It is apparent that when this practice has been followed the amount of sugar contained in a watered juice is easily estimated from the amount of added sugar solution determined by the method described previously by the following formula:

$$S = \frac{20 W}{100},$$

wherein  $S$  = grams of added sugar per 100 cc., and

$W$  = percentage by volume of added sugar solution.



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